GENETICS

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THE ANALYSIS OF CONTINUOUS VARIATION IN A DIALLEL CROSS OF NICOTIANA RUSTICA VARIETIES

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I. THE ANALYSIS OF F_1 DATA

INTRODUCTION

STATISTICAL methods for the analysis of continuous variation have been developed using second degree statistics such as variance and covariance and successfully applied to crosses between two inbred lines. These have allowed the recognition of the more familiar phenomena associated with Mendelian genetics although they appear in the analyses in a new and less obvious form (MATHER 1949; BATEMAN and MATHER 1951; MATHER and VINES 1953). The methods can be used over the whole range of mating systems employed in plant and animal breeding, selfing, sib mating, assortative mating, and can also be adapted to randomly mating groups.

In the present paper this approach has been extended to the analysis of data from a diallel cross between a number of inbred lines. The application of this method to a diallel cross in *Nicotiana rustica* and published results of similar crossing programs in maize (JINKS and HAYMAN 1953) show it to be an efficient means of obtaining a rapid, overall picture of the genetical control of a character in a number of inbred lines; at the same time throwing considerable light on the genetical basis of heterosis in the F_1 progeny of these lines.

MATERIAL

The inbred lines used in the diallel cross were varieties of *N. rustica* collected by PROFESSOR K. MATHER while at the John Innes Horticultural Institution. The advantages of these are of a technical nature, e.g., ease of selfing and emasculating. The most important advantage, however, from the point of view of the present experiment is the great range of continuous variation within the species. A number of major gene differences have been recorded mainly as a check on pollination and segregation but also in the hope that linkage studies between polygenically and major genically controlled differences may be followed in later generations. These include the genes for anthocyanin and type of inflorescence (*A-a* and *M-m* respectively) already described by MATHER and VINES in addition to duplicate factors which control the color of ovary and flower (*yo*) and a gene responsible for seed color (*B-b*) i.e., brown-black or yellow.

The eight varieties used in these experiments are 2, 5, 7, 12, 14, 29, 38 and 41, using the numbers ascribed to them by MATHER and VINES.

EXPERIMENTAL PLAN

In 1950 crosses were made between the 8 varieties in all possible combinations, including reciprocals. (Although 9 lines were originally included in the experiment, the crosses in one line, variety 31, differed from the rest in showing a significant difference between reciprocals. In consequence, variety 31 and its crosses have been excluded from the main diallel experiment and will not be discussed further here.) The 56 F_1's and the 8 parental lines were grown in 1951. Plant height, flowering time, leaf length, width and distance of maximum width from the leaf apex were recorded. A further character, the total number of seed capsules per plant was also recorded for the four parental lines 5, 12, 14 and 41 and for the 12 F_1's between them.

The families were started in a glasshouse and were later planted out in a randomized layout of two blocks in the field. Each block consisted of 80 plots (i.e., 9 lines and all possible F_1's, except that 2×41 was omitted as it gave no viable seed). Each plot consisted of 5 plants in a row. Taking both blocks into account, 10 plants were grown of each family. All rows were set 2 feet apart and plants 1 foot apart within the rows. The whole experiment was surrounded by a guard row of plants which were not scored so as to avoid edge effects. The two blocks were identical except that the plots within them were randomized separately.

The F_1's grown in 1951 were selfed to give F_2 seed and also backcrossed reciprocally to their respective parental lines. The diallel cross between the parental lines was also repeated. In 1952 the layout again consisted of two

TABLE 1

The means and variances of the diallel crosses averaged over blocks and reciprocals for height 1951. The means occupy the upper right half of the table and the variances the lower left.

		Means							
Parents		2	5	7	12	14	29	38	41
Variances	2	38.5 6.0	51.3	40.4	67.1	40.9	40.3	41.8	38.8
	5	4.1	39.3 4.1	44.6	50.0	45.6	50.5	42.4	42.7
	7	8.6	3.2	37.2 4.7	56.0	39.1	41.7	33.3	37.1
	12	7.9	5.4	13.3	52.9 9.1	54.3	60.7	49.1	52.9
	14	10.7	11.6	5.7	11.7	38.3 15.6	40.3	33.8	30.7
	29	8.4	17.4	4.2	9.0	8.9	44.3 4.4	34.9	40.6
	38	7.4	6.5	8.0	19.9	4.2	5.8	30.7 1.5	33.1
	41	7.7	10.5	3.9	11.3	5.9	25.4	5.1	37.1 25.9

TABLE 2

The means and variances of the diallel crosses averaged over blocks and reciprocals for height 1952. The means occupy the upper right half of the table and the variances the lower left.

		Means							
Parents		2	5	7	12	14	29	38	41
Variances	2	33.5 1.6	51.0	39.1	62.9	40.2	36.1	34.6	37.3
	5	9.3	38.3 10.4	39.2	51.8	44.7	48.6	40.8	43.3
	7	5.2	5.5	27.4 4.4	55.6	39.5	36.7	35.4	35.2
	12	28.9	5.9	7.0	51.3 10.6	53.2	57.2	50.6	52.5
	14	4.3	9.4	3.9	4.9	35.8 5.8	41.0	36.4	37.7
	29	3.3	7.3	2.8	10.2	4.6	39.5 8.2	35.3	37.6
	38	8.4	8.7	8.5	8.3	3.9	6.5	35.9 1.0	33.7
	41	5.2	14.0	10.3	9.0	4.6	6.8	12.5	33.3 3.5

blocks, but with 287 plots of 5 plants per block. Since line 31 and its crosses had not been included, these 287 plots were made up of 8 parental lines and 55 F_1's (the family 2×14 being omitted as no viable seed was obtained) of 1 plot per block, 56 F_2's of 2 plots per block and 108 backcrosses of 1 plot per block.

ANALYSIS OF MEANS

The method of recording height and flowering time was the same as in MATHER and VINES' experiment. In the current experiment the time of flowering is expressed in days after July 1st in 1951 and after June 20th in 1952. These dates are chosen quite arbitrarily, the earlier date being used in 1952 so that all the figures would be positive. This in no way affects the subsequent analyses, or their results.

The leaf characters were measured to the nearest half centimeter. The largest leaf on each plant, usually one of the first four leaves to be formed in these varieties, was chosen and the length from apex to the insertion of the petiole, the maximum width and the distance of this point of maximum width from the leaf apex were measured.

Because of the large numbers involved (100–400 capsules per plant) and the time taken to measure such a character, it was only recorded for 4 parental lines and the 12 F_1's between them.

The mean heights for the 8 parental lines and the 56 F_1 combinations for the seasons 1951 and 1952 are given in tables 1 and 2. These results are averaged over two blocks and reciprocal families within each block.

(i) *Combination and correlation of characters*

In order to investigate the possibility of obtaining a discriminant function for the three leaf measurements, the variances within and between families were calculated for each of the three characters in 1951.

The ratio of these two variances was almost uniform for the three leaf characters. Furthermore these characters are highly correlated, e.g., the correlation coefficient for leaf length and width is 0.8819. It appears, therefore, that the three measurements are contributing much the same information so that little is to be gained by combining them into a discriminant function.

The correlation coefficients between height and flowering time and height and leaf length for 1951 are not significant. So far as these data go, these characters are independent of one another. Height and number of capsules per plant are, however, highly correlated. Capsule number, leaf width and position of maximum width, were consequently not scored in 1952.

(ii) *Reciprocal differences*

A comparison of the means of reciprocal F_1 families has been made for all the characters both in 1951 and 1952 using the analysis described by YATES (1947). In 1951 there were no significant reciprocal differences after excluding the progenies of parent 31 (mentioned earlier). Even when the progenies of this parent were not included, however, flowering time in 1952 gave significant reciprocal differences ($P = 0.01$–0.001), while height and leaf length were significant at the 5% level. Unlike the reciprocal differences encountered in the progenies of parent 31 in 1951, those of 1952 could not be traced to the progenies of one or a small number of parental lines: they were sporadically distributed throughout the F_1 progenies of all the parental lines. They could be traced, however, with some confidence to the history of the plants prior to planting in the field. Owing to the peculiarities of the 1952 season, the young plants were kept under glass in boxes up to a much later stage than is normal. Reciprocal families being in different boxes suffered to different extents from the resulting overcrowding and eventual setback when they were ultimately planted in the field. Furthermore, flowering commenced within a few weeks of planting out, as compared with the usual period of about two months. As a result, duplicate plots in the two blocks, grown from plants from the same box, although more widely separated in the field than reciprocal families within the same block, were more alike, so giving rise, one assumes, to the significant reciprocal differences. Flowering time, scored before the plants recovered from the setback, was affected most, while height and leaf length, which are scored at the end of the growing season, were less upset, and indeed show barely significant reciprocal differences ($P = 0.05$).

In the absence of significant reciprocal differences, the mean sum of squares of reciprocal differences is our estimate of E_2 (MATHER 1949), i.e., the environmental component of the variances of family means. In the presence of reciprocal differences of the type found for the 1952 flowering time, E_2 can be obtained as the variance of differences of duplicate plots between the two

blocks. When this course is followed, the analysis to be described later must be carried out on the means of reciprocal families within each block.

(iii) *Heterosis*

An indication of the overall direction of the deviation of the F_1 means from their corresponding mid-parents can be obtained by comparing the mean of all parental lines with that of all F_1's. In all characters except number of capsules and flowering time the F_1 mean is larger. This difference can be tested for significance using an empirical error variance derived from the variation of the deviations of each F_1 family mean from its corresponding mid-parent, taking sign into account, around the overall mean deviation. This method in all cases gives high significance for the mean deviations (table 3).

It must be emphasized that these results only represent the overall direction of heterosis for any character. The significant positive heterosis for height, for example, does not exclude the possibility that individual F_1 families may show significant negative heterosis or no heterosis. Indeed examination of the results show that such families exist, although less frequently than families that show positive heterosis.

TABLE 3

Overall parental and F_1 means and the significance of the deviation (F_1 mean − Parental mean) for the characters scored over the two years.

	Height		Flowering time		Leaf length	
	1951	1952	1951	1952	1951	1952
Parental mean	39.62	36.85	30.39	18.15	20.69	21.16
F_1 mean	43.50	42.55	26.19	16.54	21.95	22.38
(F_1 − P) mean	+3.88	+5.70	−4.20	−1.61	+1.26	+1.22
Significance	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

ENVIRONMENT-GENOTYPE INTERACTION

The variance of the five plants within a plot around the plot mean has been obtained for each of the characters scored over both years. These variances, averaged over blocks and reciprocals, are given for height in tables 1 and 2. In a small number of cases these variances are based on less than 4 degrees of freedom due to the death of an occasional plant. In no case, however, are they based on less than 2 degrees of freedom, the average being 3.9. In so far as the parental lines are homozygous (and there is nothing to lead us to believe that they are not) these variances are due entirely to non-heritable effects, i.e., the E_1 component of the variance of segregating families (MATHER 1949).

The STEVENS test of homogeneity of variance (FABERGÉ 1936) when applied to these variances gives high significance ($P < 0.001$ in all cases) for heterogeneity for all the characters scored over both years. The result is the same whether duplicate families in the two blocks are averaged or analyzed independently. The non-heritable variances are thus not uniform over families.

Since there was the possibility that the heterogeneity of the variances could be removed by a suitable change of scale, the relationship between the variances and means of families were investigated to see whether low means and variances and high means and variances were associated. Scatter diagrams of the means and variances showed no obvious relationship, and the regression, which was calculated in the case of height in 1951, proved to be non-significant (P = 0.70–0.60). No simple transformation of the data would thus make the variances homogeneous.

To see whether the heterogeneity could be traced to each genotype having its own specific environmental interaction, an analysis of variance was carried out on the non-heritable variances as illustrated below for flowering time 1951. (This analysis includes the progenies of parent 31).

		SS	DF	V	VR	P
genotype	♂ arrays	8,231.7710	8	1,028.9714	3.4	0.01–0.001
	♀ arrays	9,136.7459	8	1,142.0932	3.8	0.01–0.001
	blocks	6.5214	1	6.5214	0.02	Not sig.
	♂ × ♀ arrays	27,112.8553	64	423.6384	1.4	Not sig.
	blocks × ♂ arrays	2,706.3355	8	338.2919	1.1	Not sig.
	blocks × ♀ arrays	1,311.4206	8	163.9276	0.5	Not sig.
	blocks × ♂ × ♀ arrays (error)	19,398.3828	64	303.0997		
	Total	67,904.0325	161			

Analysis of the other characters gave essentially the same results, i.e., only the male and female lines used for the F_1's have any significant effect on the size of the error variance. One could, therefore, predict the error variance of each F_1 as the sum of two constants characteristic of its two parents. The variation, or heterogeneity in the non-heritable variances is thus attributable to the varying reaction of the different genotypes to environmental differences.

Certain differences between the flowering time as just analyzed and the other characters are worth noting. For height in 1951 the male and female array sums of squares were only significant if pooled to give a genotype sum of squares for 16 degrees of freedom (P = 0.05–0.01). The block × female sum of squares was also significant (P = 0.05–0.01), but was no longer significant if pooled with the block × male sum of squares to give block × genotype sum of squares (P = 0.10–0.05). In height 1952 the female array sum of squares was significant ($P < 0.001$), but the male array sum of squares was not. Jointly, the genotype sum of squares for 14 degrees of freedom was significant ($P < 0.001$). Neither of the block interactions was significant (P = 0.10–0.05). In the leaf analysis 1951 the male array sum of squares was significant (P = 0.05–0.01), but the female array sum of squares was not, neither were any of the interactions. The summed male and female array sum of squares was also not significant (P = 0.10). This is almost certainly the result of a larger relative error in choosing and measuring the largest leaf inflating the error sum of squares. The number of capsules gave significance for the

TABLE 4

The variance within plots of all parents and all F_1's in 1951 and 1952.

Character	Parents		F_1's		Averages of both years	
	1951	1952	1951	1952	Parents	F_1's
Height	6.8200	5.6656	6.0734	7.7583	6.2428	6.9160
Flowering time	19.0806	16.6375	10.4790	14.1219	17.8591	12.2705
Leaf length	3.3066	2.2907	2.1495	3.8967	2.7987	3.0231

male array sum of squares (P = 0.01) and the joint genotype sum of squares (P = 0.01–0.001) but not for the female array sum of squares (P = 0.1–0.05).

Despite these minor disturbances there can be little doubt that different genotypes show different amounts of non-heritable variation; or, to put in another way, different genotypes respond in different ways to given changes in environmental conditions.

In table 4 are given the mean variance within plots of all parents and all F_1's for the characters scored over the two years.

Only in the case of flowering time does the difference in variability in parents and F_1's approach significance and then only at the 5% level. For the other characters the difference is neither significant nor consistent in sign over the two years. Overall there appears to be very little difference in the response of the homozygous parents and heterozygous F_1's to differences in the environment.

SECOND DEGREE STATISTICS

The expected variances and covariances obtainable from the parents and progenies of a diallel cross can be found by the methods of MATHER (1949). Let us consider one locus represented by two allelomorphs A and a in the parental lines such that a proportion u_a of the parental lines are AA and $v_a(= 1 - u_a)$ are aa. The array of gametes will then be u_aA and v_aa. If the overall mid-parent is taken as 0 and A adds on $+d_a$ and a, $-d_a$, while the heterozygote deviates by h_a from the mid parent, then in a diallel set of crosses the contribution of this locus to the means of the families will be as follows.

Parents	Genotype	AA	aa	array
Males	Frequency	u_a	v_a	means
Females	Mean	d_a	$-d_a$	
AA u_a d_a		AA u_a^2 d_a	Aa u_av_a h_a	$u_ad_a + v_ah_a$
aa v_a $-d_a$		Aa u_av_a h_a	aa v_a^2 $-d_a$	$-v_ad_a + u_ah_a$
Parental mean $(u_a - v_a)d_a$		Overall mean of progenies $(u_a - v_a)d_a + 2u_av_ah_a$.		

The mean variance of the F_1's in an array, i.e., all the progenies of a common parent, around the array mean is

$$\text{mean variance of an array} = V_{1L1} = u_a[u_a d_a^2 + v_a h_a^2 - (u_a d_a + v_a h_a)^2] + v_a[u_a h_a^2 + v_a d_a^2 - (u_a h_a - v_a d_a)^2]$$

This is similar to the expectations for the variance of a random mating population given by MATHER (1949), which reduces to

$$u_a v_a[d_a + (v_a - u_a)h_a]^2 + 4u_a^2 v_a^2 h_a^2.$$

For a number of independent genes, i.e., genes showing neither interaction nor non-random distribution in the parental lines, this becomes

$$V_{1L1} = \tfrac{1}{4}D + \tfrac{1}{4}H + E_2.$$

Where $D = 4\Sigma uv(d + (v - u)h)^2$ and $H = 16\Sigma u^2 v^2 h^2$ as defined by MATHER 1949 for random mating systems. The component E_2 is included to allow for the non-heritable variance of means due to effects of the environment.

Similarly the contribution of the gene *A-a* to the variance of array means around the overall progeny mean is given by

$$u_a(u_a d_a + v_a h_a)^2 + v_a(u_a h_a - v_a d_a)^2 - [(u_a - v_a)d_a + 2u_a v_a h_a]^2$$

and summing over all independent genes we find $V_{0L1} = \tfrac{1}{4}D$. Now this will have a non-heritable component E_3 which is $1/n$ E_2, where n is the number of families in the array, i.e., the number of parental lines.

The overall variance of progeny means around the overall progeny mean is, of course, the sum of the above two variances, i.e., $\tfrac{1}{2}D + \tfrac{1}{4}H$, the non-heritable factor again being E_2. When $u = v = \tfrac{1}{2}$, the overall variance of the diallel crosses is the same as the variance of an F_2 family from a cross between two inbred lines.

In addition to these variances one can also obtain the covariance of offspring on parents. For a diallel cross there are two methods of obtaining this covariance, viz., the covariance of array means on the common parent of the array and the mean covariance of members of an array on the non-common parents, both of which reduce to

$$2u_a v_a d_a[d_a + (v_a - u_a)h_a].$$

Unlike the variances obtainable from the diallel cross it is impossible to express this covariance in terms of D and H appropriate to the variances and covariances of a random mating population. It is necessary, therefore, to redefine our D for the covariances as $D_W = \Sigma 4uvd[d + (v - u)h]$. Each term of this D is the geometric mean of the corresponding terms of the random mating D and $D(= \Sigma d^2)$ of a one by one cross between inbred lines, though of course this property does not hold for the D's themselves. Covariances normally have no non-heritable component (E) since the pairs of measurements, which give the cross-products from which covariances are calculated, are as likely to be affected in opposite ways as the same way by non-heritable agencies. In diallel crosses, however, each parental value occurs also as a member

of its own progeny array, hence the covariance of offspring on parents contains square terms as well as cross products, the former having a non-heritable component that does not cancel out. In an $n \times n$ diallel cross, of the n^2 progenies, n are of this type, so that the covariance has a non-heritable component $1/n^{th}$ of the mean variance of arrays, i.e., E_3.

When reciprocal F_1 families are grown two methods of analyzing the data are available in the absence of any significant reciprocal differences. Firstly, all the statistics may be calculated for both male and female arrays or their array means and then averaged. In this case the non-heritable components have the coefficients given above. Alternatively, reciprocal F_1 families may be averaged prior to the calculations, this method being essential when significant reciprocal differences are present. This necessitates adjustments in the coefficients of some of the non-heritable components as each F_1 cross is the mean of two plots while each parental mean is represented by only one plot. If n equals the number of parental lines in the diallel cross, the E components of the variance and covariance are:—

$$V_{1L1}, \quad \frac{n+1}{2n}E_2 \qquad V_{0L1}, \quad \frac{1}{2n}E_2 \qquad \text{and} \qquad W_{0L01}, \quad \frac{1}{n}E_2$$

Table 5 gives the estimates of these three statistics, corrected for the appropriate E components, along with the estimate of E_2 from which these corrections have been calculated. These estimates have been found separately for each block and then averaged.

TABLE 5

Estimates of the second degree statistics obtainable from a diallel cross for the N. rustica experiment.

Character		Statistic						
		V_{1L1}	V_{0L1}	W_{0L01}	D	D_W	H	E_2
Height	1951	49.3107	31.5812	34.0085	126.3248	68.0170	70.9180	2.4885
	1952	48.0110	28.5165	31.5141	114.0660	63.0282	77.9780	7.7086
Flowering time	1951	22.0089	13.0203	34.7186	52.0812	69.4372	27.9544	2.5609
	1952	13.2525	7.8853	10.0018	31.5412	20.0036	21.4688	3.3738
Leaf length	1951	5.5397	4.3739	7.4065	17.4956	14.8130	4.6632	2.1706
	1952	3.7602	3.8592	6.8291	15.4368	13.6582	−0.3960	2.8814

No estimates of error are given as only exact fit to observation can be obtained from the number of statistics available, and the estimate from block differences, if obtained, would only be based on one degree of freedom.

Comparison of H with either D or D_W gives little idea of the average degree of dominance since, when $u \neq v \neq \frac{1}{2}$, D includes some effect of h, and H is correspondingly less than the summed effects of all the squared h deviations. (The relationship between D and Σd^2 has been worked out by MATHER (1949) for a range of gene frequencies in the parental lines.) A value of H,

however, of the same order as D and D_W does suggest that dominance is operative in these progenies, e.g., in height.

A better idea of the average degree of dominance can be obtained from a comparison of V_{1L1} and W_{0L01} after subtracting the environmental components. Thus V_{1L1} can be written as

$$\Sigma uv[d^2 + h^2 + 2(v - u)dh]$$

while

$$W_{0L01} = \Sigma uv[2d^2 + 2(v - u)dh]$$

Therefore if $d = h$ then $V_{1L1}/W_{0L01} = 1$, while if $d > h$ $V_{1L1}/W_{0L01} < 1$, or if $d < h$ $V_{1L1}/W_{0L01} > 1$.

For independent genes with $u = v = \frac{1}{2}$ for all the genes the graph connecting V_{1L1}/W_{0L01} and h^2/d^2 is a straight line. If $u \neq v \neq \frac{1}{2}$ the line becomes a curve but always passes through the two points ($h^2/d^2 = 0$, $V_{1L1}/w_{0L01} = \frac{1}{2}$) and ($h^2/d^2 = 1$, $V_{1L1}/w_{0L01} = 1$). This estimate of the average degree of dominance has one advantage over the other estimates in that it does not suffer from errors arising from partitioning D and H which are correlated in all the available statistics. The ratio of V_{1L1}/W_{0L01} is given for the various characters in tables 6 and 8. Discussion of these ratios will be reserved until later when other estimates of dominance are given.

A further useful second degree statistic can be obtained from diallel crosses, namely the variance of the parental means around the mid parent. The expectations for this statistic in terms of a single gene are

$$\begin{aligned} V_{0L0} &= u_a d_a^2 + v_a d_a^2 - [(u_a - v_a)d_a]^2 \\ &= 4u_a v_a d_a^2. \end{aligned}$$

Summing over a number of independent genes this becomes

$$\Sigma\, 4uvd^2.$$

This of course has a non-heritable component E_2.

Now terms in $\Sigma\, uvd^2$ are present in all the available statistics. Furthermore, this new statistic provides sufficient equations for obtaining an independent estimate of $\Sigma\, dh$ terms, leaving terms in $\Sigma\, uvd^2$ and $\Sigma\, uvh^2$, which, having similar coefficients, are directly comparable. D can now be defined to cover both variances and covariances as $4\Sigma\, uvd^2$, H_1 as $4\Sigma\, uvh^2$, $H_2 (= H)$ as $16\Sigma\, u^2v^2h^2$ and F as $8\Sigma\, uv(u - v)dh$, the last being the only statistic that can take sign.

The compositions of the variances and covariance in terms of the newly defined D, H_1, H_2 and F are

$$\begin{aligned} V_{0L0} &= D \\ V_{1L1} &= \tfrac{1}{4}D + \tfrac{1}{4}H_1 - \tfrac{1}{4}F \\ V_{0L1} &= \tfrac{1}{4}D + \tfrac{1}{4}H_1 - \tfrac{1}{4}H_2 - \tfrac{1}{4}F \\ W_{0L01} &= \tfrac{1}{2}D - \tfrac{1}{4}F. \end{aligned}$$

If $u = v = \frac{1}{2}$, D becomes Σd^2 and $H_1 = H_2 = \Sigma h^2$ as obtained for a cross between two inbred lines (MATHER 1949).

The estimates of these parameters are given in table 6 for height 1951 and 1952 along with the estimates of dominance H_1/D and V_{1L1}/W_{0L01}.

Again there is no useful estimate of error, since five parameters (including E_2) are estimated from five equations. Nevertheless, the consistency of all the statistics over years and over blocks, within years (not given in table) allows one to interpret these results with confidence even in the absence of standard errors of the components.

Both the ratios H_1/D and V_{1L1}/W_{0L01} suggest that overdominance is present, i.e., H_1/D and $V_{1L1}/W_{0L01} > 1$, but it must be remembered that these ratios are measuring dominance on different scales, although they can be converted to the same scale when $u = v = \frac{1}{2}$. H_1/D then becomes $\Sigma h^2/\Sigma d^2$ while V_{1L1}/W_{0L01} becomes $\Sigma h^2/\Sigma 2d^2 + \frac{1}{2}$. Thus subtracting half from the ratio V_{1L1}/W_{0L01} and doubling the remainder should lead to a value approaching H_1/D. In the absence of information concerning u and v this transforma-

TABLE 6

Estimates of the parameters D, H_1, H_2 and F and dominance H_1/D and V_{1L1}/W_{0L01} for height 1951 and 1952, including and excluding arrays 1, 2 and 4.

	Year	Parameters					
		D	H_1	H_2	F	H_1/D	V_{1L1}/W_{0L01}
All arrays	1951	42.3092	103.5180	70.9178	−51.4151	2.4467	1.45
	1952	43.0078	108.9958	77.9780	−40.0402	2.5343	1.68
Omitting arrays 1, 2 and 4	1951	19.5548	11.8878	11.1522	− 6.1518	0.6079	0.8306
	1952	22.0921	19.0100	9.7440	+18.0896	0.8605	0.8716

tion has in all cases given results that agree more closely with H_1/D, e.g., height 1951, $H_1/D = 2.45$, $2(V_{1L1}/W_{0L01} - \frac{1}{2}) = 1.90$, 1952, $H_1/D = 2.53$, $2(V_{1L1}/W_{0L01} - \frac{1}{2}) = 2.36$.

RELATIONSHIP BETWEEN ARRAY VARIANCE AND ARRAY COVARIANCE

Returning to our consideration of a single gene we find that the variance of the *AA* array (V_{AA}) is $u_a v_a (d_a - h_a)^2$ and the covariance (W_{AA}), $2u_a v_a d_a (d_a - h_a)$. Similarly for the *aa* array, $V_{aa} = u_a v_a (d_a + h_a)^2$, while $W_{aa} = 2u_a v_a d_a (d_a + h_a)$. Thus substituting *aa* for *AA* changes both V and W by the same amount, $4u_a v_a d_a h_a$; or to put it in another $V_r - W_r$ is constant over arrays. Extending this to a number of independent genes gives the same result, viz., that $V_r - W_r$ is constant. The regression of array covariance and array variance should therefore give a straight line of slope 1. The only limiting condition is that each gene should be independent of all others in its action. It should therefore be possible to detect genic interaction by deviations from this expected regression line.

A further property of this regression line can be utilized in the study of

the dominance relations. When the variance of an array is zero, its covariance is $\frac{1}{4}D - \frac{1}{4}H_1$, i.e., $W_r - V_r = \frac{1}{4}D - \frac{1}{4}H_1$. Now we have a test of significance to see whether the regression line passes through the origin, i.e., whether D is greater than, equal to, or less than H_1 in value, so that we can test for significant overdominance, complete dominance or underdominance. Furthermore, if there is significantly less than complete dominance, it is possible to test whether H_1 is significantly different from zero, since if $H_1 = 0$, there is no significant regression, i.e., the variances and covariances of all arrays are identical, within experimental error, being estimates of a single point where $W_r/V_r = 2$ (fig. 1).

Before proceeding to analyze the data we will first examine the ways in which genic interaction might be expected to result in deviations from a uniform regression of unit slope. Interaction affecting a proportion of the

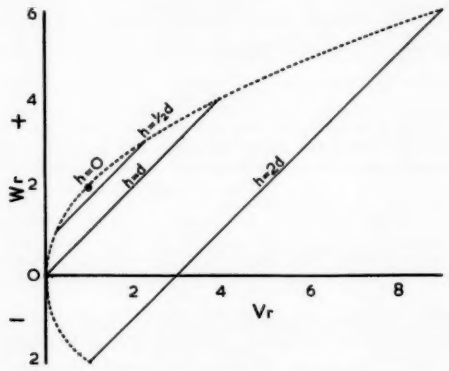

FIGURE 1.—The theoretical regressions of W_r on V_r for various degrees of dominance, i.e., h/d ratios. The curve (broken line) joins the points of the arrays whose common parents contain all the dominant or all the recessive allelomorphs.

members of an array will increase the variance of that array while the parent offspring covariance will fall. Examination of figure 1 shows that any increase in variance relative to covariance will move the W_r, V_r graph to the right giving an apparent increase in dominance. At the same time, this increase in V_r relative to W_r will have a proportionately greater effect as we move from the point of origin along the regression line. As a result not only will the mean of the regression move towards the right (indicating higher dominance) but its slope will fall below the expected value of one.

The extent to which this happens and hence the ease with which it can be detected, from its effect on the slope, will depend on the ratio of interacting to non-interacting members in the arrays. When this ratio is high, no difficulty, of course, will be met with. If, however, it is low, the deviation of the few array points affected by interaction may serve only to inflate the error variance of the regression line to such an extent as to obscure the deviation

from unit slope. The latter case may be dealt with by omitting those arrays which lead to an improvement of the average slope and at the same time reduce the standard error of the regression coefficient.

The 1951 height data gave a regression coefficient of 0.4079 ± 0.04611 and the 1952, 0.5963 ± 0.1282, both of which differ significantly from a slope of one (fig. 2). There was no block × regression interaction, neither were there differences between block means in either sets of data. Examination of the graph of W_r against V_r showed that in both years the arrays fell into two groups. Most of the points fell on a straight line of slope 0.6–0.7, while three points for arrays 1, 2 and 4 (common parents, 2, 5 and 12) deviated more than the rest from a slope of one. This is compatible with an incidence of interaction highest in arrays 1, 2 and 4 but also occurring to a smaller extent

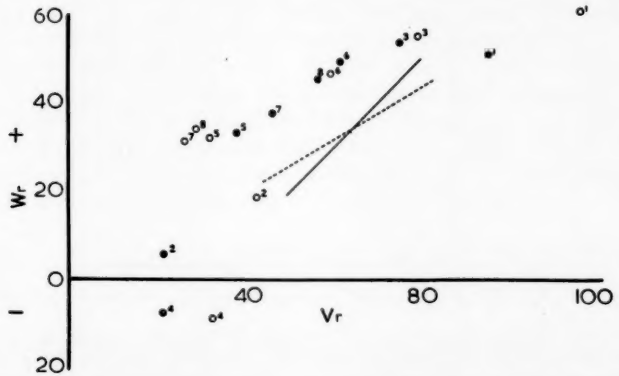

FIGURE 2.—The regression of W_r on V_r for height 1952. The best fitting regression line has a slope of 0.5963 ± 0.1282 (broken line). The complete line is the theoretical slope of 1 expected if there is no genic interaction. The points for arrays 1, 2 and 4 obviously deviate most from the best fitting line. The points for the duplicate blocks are differentiated by using full and open circles.

in the other arrays, possibly being confined to the families in these arrays having either 1, 2 or 4 as one parent. If this is correct then the removal of the progenies of 1, 2 and 4 from the data should result in the remainder fitting a regression coefficient of 1.

In the 1952 data this proved to be the case (fig. 3). Removal of the progenies of parents 1, 2 and 4, and only these three, gave a regression coefficient of 0.7806 ± 0.2217, which is not significantly different from a regression coefficient of 1. In the 1951 data, however, removal of the progenies of parents 1, 2 and 4 still did not account for all the genic interaction, the regression still being less than 1 ($P = 0.02$–0.01). Examination of the graph of W_r against V_r after removing the progenies of 1, 2 and 4 showed that apart from array 8 (common parent 41) all the other arrays fell on a straight line of approximately unit slope. This remaining interaction could be traced to the F_1 family

5×8 since the removal of the progenies of either 5 or 8, while not affecting the other array points, brought the alternative array into line. Furthermore omitting the array points of 5 and 8 but leaving their progenies in the other arrays gave a regression coefficient that was not significantly different from 1 ($b = 0.6878 \pm 0.2790$). The 1951 results agree therefore quite well with those of 1952.

It must be borne in mind that although removal of the progenies of arrays 1, 2 and 4 removes all the genic interaction in 1952 and most of it in 1951, it must obviously be possible to remove interaction by omitting the progenies of the parents with which 1, 2 and 4 interact in the F_1's. Since, however, the intensity of interaction is highest in these three arrays, removal of the interaction by the alternative means would presumably require the omission of a larger number of arrays.

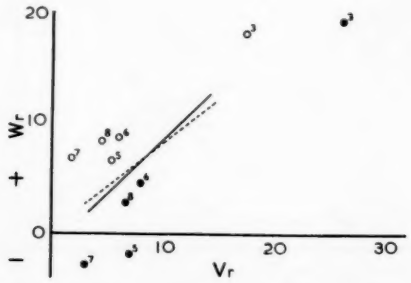

FIGURE 3.—The regression of W_r on V_r for height 1952 after removing the progenies in arrays 1, 2 and 4, i.e., the offspring of parents 2, 5 and 12. The best fitting line now has a slope of 0.7805 ± 0.2217, which does not differ significantly from the theoretical slope of 1 (the complete line). The array points are arranged along this line in the order of the number of dominant allelomorphs in their common parent, array 3 having least and array 7 the most. The points for the duplicate blocks are differentiated by using full and open circles. The slopes for the two blocks have significantly different means, i.e., different degrees of dominance in the two blocks.

We can now examine in the residual progenies the means and second degree statistics for height free from complications arising from the presence of interaction in the data. Unfortunately, in removing the interacting arrays the number of families has fallen from 64 to 25, hence the analysis is not so comprehensive nor the agreement between years so good as in the complete data. Nevertheless, certain consistencies are obvious over the two years. Firstly examination of the tables of progeny means (tables 1 and 2) shows that the removal of arrays 1, 2 and 4 (common parents 2, 5 and 12) removes all the families whose means are higher than their own better parent mean. Furthermore, parents 5 and 12 give good F_1 families with the same other parents but not with each other, while parent 2 gives high means with both 5 and 12 and with a number of other parents with which 5 and 12 give mediocre F_1's. Thus most of the parents give good F_1 families with either 2 or 5 and 12.

This agrees remarkably well with the regression analysis if we accept the view that the high means are the result of genic interaction of a type comparable with the complementary genic action of classical genetics. It would appear, therefore, that in these crosses heterosis is not the result of overdominance but of complementary gene interaction.

Secondly if we now examine the estimates of the second degree parameters D and H_1 from the residual progenies we find that the " overdominance " has disappeared and that we now have slightly less than complete dominance in both years (table 6).

If the deviation of $\frac{1}{4}D - \frac{1}{4}H_1$ from 0 is tested by the regression method we find that in both years there is no significant deviation from complete dominance. While admitting the possibility that the three arrays which were removed as the main source of genic interaction might well be the only arrays that also show overdominance, it would appear to be more reasonable to assume that the drop in apparent dominance is due almost entirely to the removal of the interaction.

A further test of interaction, though one not so clear as that afforded by the regression of W_r on V_r because of other disturbances it also detects, has been devised in collaboration with PROFESSOR K. MATHER. If we subtract half the deviation of the common parent of an array from the overall mid-parent, from the array mean the following expectations are obtained:

Array	array mean − ½deviation of common parent from parental mean
AA	$u_a d_a - v_a h_a - \frac{1}{2}u_a d_a = \frac{1}{2}u_a d_a + v_a h_a$
aa	$-v_a d_a + u_a h_a + \frac{1}{2}v_a d_a = \frac{1}{2}u_a d_a + u_a h_a$

If $u_a = v_a = \frac{1}{2}$, then these expectations will not differ significantly from one another unless there is genic interaction. Under these conditions the values will be homogeneous when tested against an error variance found as $E_2(1 + 1/n)$, where n = number of families in each array, as a χ^2 for n degrees of freedom. Homogeneity may break down when $u \neq v$, but even then the removal of any interaction which is present should at least lead to a significant lowering of the heterogeneity sum of squares, unless of course removing the interaction seriously disturbs the u, v distribution.

Applying this F_1 scaling test to the 1951 height data gives a heterogeneity SS of 86.3792 for 8 degrees of freedom which is significant when compared with the error MS of 1.0790. After removing the progenies of arrays 1, 2 and 4 the heterogeneity SS drops to 4.3538 and the error MS becomes 1.2450, so that the remaining progenies are homogeneous (P = 0.8–0.7). Hence this method, within its limitations, detects the same sources of interaction or heterogeneity as the regression analysis.

THE RELATIVE FREQUENCIES AND DISTRIBUTION OF ALLELOMORPHS IN THE PARENTAL LINES

An estimate of the average value of the product uv for all the gene differences in the parental lines can be obtained from the ratio $\frac{1}{4}H_2/H_1 =$

$4\Sigma u^2v^2h^2/4\Sigma uvh^2$. This, of course, will be subject to an error depending on the standard errors of H_1 and H_2 which are unknown. Since this estimate of $\overline{uv}$ is obtained solely from terms in Σh^2 it will of necessity only cover the frequencies of the allemorphs of genes that are exhibiting some degree of dominance. Thus it provides no evidence about the distribution of allelomorphic pairs exhibiting no dominance. The results for height 1951 and 1952 are $\overline{uv} = 0.1713$ and 0.1788 respectively.

The sign of $F[\Sigma 8uv(u-v)dh]$ depends on the sign of $(u-v)h$ which is positive for an excess of dominant allelomorphs and negative for an excess of recessives. The sign of F therefore is an indicator of the relative frequencies of dominant and recessive allelomorphs. For height in both 1951 and 1952 F was negative, hence there is greater frequency of recessive than dominant allelomorphs in the parental lines.

If we examine figure 1 we find that the ratio of W_r/V_r for the complete dominant and complete recessive array is characteristic of the degree of dominance (table 7). By complete dominant and complete recessive array we

TABLE 7

The relationship between the covariance and variance of the complete dominant and complete recessive arrays for different degrees of dominance.

Degree of dominance h/d	W_r/V_r	
	Complete dominant array	Complete recessive array
0	2.0	2.0
½	4.0	1.3
1	0.0	1.0
2	−2.0	0.6

mean arrays whose common parents carry all the dominant and recessive allelomorphs respectively, whether these are in the direction of increase or decrease in the size of the character under consideration.

Thus given the degree of dominance from the H_1/D, V_{1L1}/W_{0L01} or $2(V_{1L1}/W_{0L01} - ½)$ it is possible to see whether the complete dominant and complete recessive arrays are present in the data. Further, if we assume that all the gene differences are equal, i.e., $d_a = d_b = d_c \ldots d_n$, then the other arrays will be scattered along the regression line, connecting array covariance with array variance, between the complete dominant and recessive arrays, in proportion to the relative numbers of dominant and recessive allelomorphs in the common parent of the array (figs. 2, 3 and 4).

NUMBER OF EFFECTIVE FACTORS

So far this discussion has been in terms of individual genes but this terminology has been solely for ease of description. In biometrical genetics we are, of necessity, forced to work in terms of the average effects of all the gene differences affecting the character under consideration. These may, however, be resolved into smaller groups of genes whose effects are statistically separa-

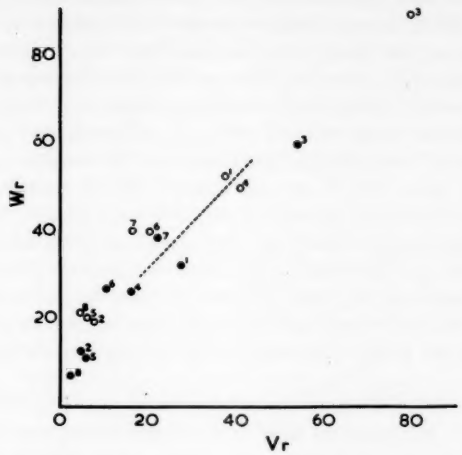

FIGURE 4.—The regression of W_r on V_r for flowering time 1951. This is almost a perfect fit with the regression slope of 1 expected in the absence of genic interaction. The array points are distributed along the regression line in the order of the number of dominant allelomorphs carried by their common parent, array 3 having least and array 8 the most. The points for the duplicate blocks are differentiated by using full and open circles.

ble on certain assumptions and whose numbers are thus capable of estimation. These smallest units of hereditary material recognizable in biometrical genetics are referred to as effective factors, which may or may not be synonymous with single genes in any particular case (MATHER 1949). In the estimations of the number of effective factors from crosses between two inbred lines described by MATHER tightly linked genes appear as one effective factor while independently segregating genes appear as separate effective factors. In the estimates from F_1 data described below there is a comparable disturbance resulting from the association of genes in the parental lines. Thus if two genes appear in all the parental lines either in reinforcement (the two d's with the same sign) or opposition (the d's taking opposite signs) then they appear as one effective factor. If, however, genes appear in all combinations in the parental lines then the apparent number of effective factors will depend on the relative frequencies of the reinforcing and opposing combinations, the maximum value being obtained when they are present in equal frequencies.

The estimate of the number of effective factors employed here is essentially the same as that described by MATHER (1949), but is based on the H, rather than the D, statistics. The difference between the overall progeny mean and the parental mean is 2Σ uvh. Therefore, the square of this difference divided by $\frac{1}{4}H_2(=4\Sigma u^2v^2h^2)$ gives an estimate of the number of effective factors

$$\text{i.e.,} \quad K = \frac{[2\Sigma uvh]^2}{4\Sigma u^2v^2h^2}.$$

Now this will be a minimal estimate for the same reasons as those given by MATHER (1949), i.e., it assumes equality and absence of oppositions in the h's for the various genes; both assumptions being unlikely to apply in practice. Unfortunately, there is no obvious way of obtaining an estimate from the D statistic which is only based on the first assumption and hence is likely to be more reliable. As in the case of the $\overline{uv}$ estimates, these estimates from the H statistics tell us nothing about genes that do not show some degree of dominance, i.e., genes that do not contribute to the H statistics.

The values of K for height are 0.85 for 1951 and 1.67 for 1952. These estimates are disappointingly small, but they are nevertheless of the same order as those obtained by MATHER (1949) from the analysis of a wide range of material. It would appear from these results that the basic assumptions on which this estimation is based are far from being realized in the present data and for that matter in any other data so far analyzed by these methods.

ANALYSIS OF THE OTHER CHARACTERS OF *N. rustica* STUDIED

A summary of the results of these analyses when applied to flowering time, leaf length and number of capsules per plant is given in table 8.

In the 1952 diallel experiment the cross 1×3 behaved anomalously for flowering time so that in the analysis it appeared as genic interaction. When the observed flowering time for this one family was discarded and a new value estimated by the missing plot technique the interaction disappeared, giving the results shown in table 8.

Although flowering time agrees in the degree of dominance and absence of genic interaction for the two years the value of D, and hence of F, differs considerably. When one considers, however, the effect of weather conditions on the rate of flowering, this year to year variation is perhaps not so surpris-

TABLE 8

Analysis of flowering time, leaf length and number of capsules in 1951 and 1952.

Statistic	Flowering time		Leaf length		No. of capsules, 1951
	1951	1952	1951	1952	
D	102.5686	16.5925	14.9831	11.3944	12,597.7664
H_1	51.7294	8.3950	5.5164	−1.7628	6,913.8168
H_2	35.9544	7.7408	4.6632	−0.7924	4,548.6752
F	+16.5657	−0.3950	−1.6590	−4.5276	−8,197.7008
H_1/D	0.5043	0.5052	0.3682	....	0.5488
V_{1L1}/W_{0L01}	0.6339	0.7555	0.7480	0.5506	0.8361
$2(V_{1L1}/W_{0L01} - \frac{1}{2})$	0.2678	0.5110	0.4960	0.1101	0.6722
F_1 scaling test	Homogeneous both years ($P = 0.10$–0.05)		Heterogeneous both years ($P < 0.001$)		Heterogeneous ($P < 0.001$)
$b\ W_r/V_r$	1.0520 ± 0.0903	0.9052 ± 0.1078	0.6871 ± 0.1820	0.7559 ± 0.1792	Not sig.
Dev. from b = 1	$P = 0.6$–0.5	$P = 0.4$–0.3	$P = 0.2$–0.1	$P = 0.2$–0.1	....
Sig. $\frac{1}{4}D - \frac{1}{4}H_1$	$D > H_1$ $P = 0.01$–0.001		$D > H_1$ $P = 0.01$–0.001	$D > H_1$ $P < 0.001$	....
$\overline{uv}$	0.1738	0.2309	0.2113	....	0.1645
K	1.96	1.77	0.055	....	0.095

ing. The genetic picture is essentially the same for the two years. Apart from the consistencies in degree of dominance and absence of genic interaction, mentioned above, we also find that the order of the array points along the V_r, W_r regression line is identical over the two years. The regression analysis further shows that $\frac{1}{4}D - \frac{1}{4}H_1$ is significantly positive for both years, so that D is significantly greater than H_1. Thus for flowering time dominance is incomplete: H_1 is shown to be significantly greater than 0 by the significant regression coefficients for W_r against V_r.

The ratio of W_r/V_r for the individual arrays suggests that the complete recessive parent is present. Thus array 3 gives $W_r/V_r = 1.12$. Taking $H_1/D = 0.5$ (table 8) then the complete recessive array should have a W_r/V_r of 1.18.

The number of effective factors is of the same order as for height.

Although the D's are of the same order for leaf length in both years the H terms show considerable divergence. Since, however, a negative H_1 and H_2 is theoretically impossible (both terms being quadratic in h), the negative values for 1952 could only arise by error variation in its effects on the negative correlation between D and H_1. The V_{1L1}/W_{0L01} and $2(V_{1L1}/W_{0L01} - \frac{1}{2})$ estimates of the degree of dominance do not suffer from such disturbances but they are nevertheless lower than the similar estimates for 1952, so suggesting that there is a genuine lower degree of dominance in the 1952 data. In both years D is significantly greater than H_1; dominance is real but incomplete.

The information from the regression analysis is remarkably consistent over the two years. Both regression coefficients fail to depart significantly from one, but examination of the graphs shows that although seven of the arrays fall on a line of slope $\simeq 0.9$, the array of parent 2 deviates considerably from this line resulting in an overall regression coefficient of $\simeq 0.7$. This suggests a high intensity of interaction confined to a small proportion of the arrays, so that the presence of the interaction is masked by the high standard error of the regression coefficient, resulting from the wide scattering of the points for the arrays showing the interaction. In this case, however, there is no difficulty in detecting the presence of genic interaction or tracing it to its source. As in the case of height, the array of parent 2 contains all the families whose leaf lengths are greater than their own best parent.

Since array 1 contributed a large proportion of the genic interaction for height, it would appear to be likely that the interaction in leaf length results from the action of the same gene complex. If this is true, then the intensity of the interaction is less marked for leaf length since it shows in only one array and not three as in the case of height.

Although the number of capsules per plant was recorded only in the 1951 experiment and then only for the four arrays 2, 4, 5 and 8 it is of interest in that this character also shows indications of genic interaction. The regression of array covariance on variance was not itself significant. The graph showed, however, that arrays 5 and 8 (parents 14 and 41) from both blocks fell on a straight line of slope approximately 0.7, while the array points for parents 5 and 12 deviated to the lower side of this line and occupied the same relative

positions as in the similar graph for height. Since only four arrays were involved, this could not be pursued further to see if the removal of the progenies in arrays 1 and 4 resulted in the remaining arrays giving a slope of 1. This similarity to height bears out the earlier observation that the means of the two characters were highly correlated.

DISCUSSION

Having tested the diallel analysis on a variety of material the results suggest that it may prove to be a powerful method for obtaining a rapid, overall picture of the genetical structure of a large number of parental lines. Although it does not allow an estimate of the standard errors of the individual statistics in its present form, the regression analysis at least makes it possible to test the significance of H_1/D, the statistic in which we are primarily interested. Furthermore, by raising the F_2 and subsequent generations by selfing each F_1 family and their progenies one can obtain sufficient statistics to allow estimates of the standard errors by the least squares method (MATHER 1949).

The regression analysis provides a simple method of detecting genic interaction which, as far as we have gone, shows good agreement with the standard scaling tests which detect non-additivity of gene action (MATHER 1949). Furthermore, this analysis has proved the presence of genic interaction in data where its presence had not been previously demonstrated although analyzed as far as already existing methods would allow (JINKS and HAYMAN 1953; JINKS 1954).

The conclusions of this analysis lend considerable support to the view that genic interaction is responsible for a large proportion of the observed heterosis in the F_1 progeny of crosses between inbred lines. It is impossible from these analyses to assess the relative contribution of other causes of heterosis, namely overdominance and the bringing together in one plant of complementary sets of genes in opposition which individually show only complete dominance or even lower degrees of dominance. One can say, however, that in the data analyzed the most outstanding F_1 families occur where genic interaction is superimposed on the other causes to give what has been termed "high combining ability."

The question of the number of effective factors is not quite so satisfactory. Firstly, our estimates must of necessity come from the H statistics which are undoubtedly inferior to those obtained from the D statistics. Secondly, any deviation from a random association of gene differences throughout the parental lines leads to a further minimizing of the estimate. In the *N. rustica* experiment the estimates never exceed two effective factors. This is undoubtedly a gross underestimate of the true position. At least two gene complexes must be postulated to explain the presence of genic interaction in height yet genic interaction is only a small proportion of the total genetical variation present as the analysis after excluding the interaction clearly shows.

Although the analysis has been applied only to diallel experiments involving plants it is equally applicable to animal breeding programs of this type. Fur-

thermore, it probably holds more advantages for the animal breeder than the plant breeder, with whom economic considerations in planning the programs do not play so important a part. The most important advantage, of course, is the time required: estimates can be obtained solely from the F_1 generations, and if confirmation of the results is required it can be supplied either from an F_2 or backcross generation. This would automatically reduce the time required to obtain estimates which is of great importance in slow maturing animals. Relieved of the burden of keeping experimental stocks for long periods and an ever expanding crossing program, the breeder can afford to use larger numbers of stocks in the initial diallel cross. In animals, of course, one would have to substitute herds for inbred lines of plants. These are less likely to be homozygous than the plant material but this would not be serious if the variation within stocks was of a much lower magnitude than that between them. The residual variation would be presumably randomly distributed throughout a herd and hence it could be investigated by using the random mating analysis (MATHER 1949). This differs from the diallel analysis only in the composition of the covariance. Herds are, however, presumably homozygous for the genes controlling the character that distinguish them and hence could be investigated by the diallel cross analysis.

As attention is increasingly turning to the possibilities of utilizing the F_1 heterosis from crosses between inbred lines as a means of increasing the yield of crop plants and animals, it is essential to gain some idea of the genetical structure of heterosis, not only from the theoretical viewpoint but also from considerations of the efficacy of choosing and testing suitable parental lines for their ability to give this heterosis. The diallel analysis described here is an attempt to provide an analytical method based on Mendelian concepts for use as a tool in investigations of this type.

SUMMARY

A method of analyzing quantitative data from diallel crosses based on the partitioning of second degree statistics, such as variances and covariances, has been developed, by extending the system described by MATHER (1949) for crosses between two inbred lines. This analysis has been successfully applied to a diallel cross between *Nicotiana rustica* varieties.

The method allows estimates of D and H_1 which are weighted terms in Σd^2 and Σh^2 respectively (MATHER 1949), so that H_1/D gives a direct estimate of the degree of dominance. The ratio of positive to negative allelomorphs in the parental lines can be obtained, as well as the ratio of dominant to recessive allelomorphs, from two further statistics, H_2 and F.

The regression of array covariance on variance has an expected slope of 1. Agreement with this expectation is found for flowering time in the *N. rustica* data. The remaining data all show varying degrees of deviation from this expected slope, the cause of which has been traced to genic interaction in some of the F_1 progenies. Removal of the progenies responsible in all cases gave the expected slope of 1.

When $V_r = 0$, then $W_r = \frac{1}{4}D - \frac{1}{4}H_1$, hence the regression analysis gives a test of significance for the level of dominance, i.e., whether W_r is equal to, greater than or less than 0 when $V_r = 0$.

Using this analysis it has been shown that genic interaction is responsible for all the apparent overdominance and heterosis in height and leaf length in *N. rustica*.

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LITERATURE CITED

BATEMAN, A. J., and K. MATHER, 1951 The progress of inbreeding in barley. Heredity **5:** 321–348.

FABERGÉ, A. C., 1936 The physiological consequences of polyploidy. J. Gen. **33:** 365–382.

JINKS, J. L., 1954 The genetical basis of heterosis. Maize Gen. Coop. News Let. **28:** 47–50.

JINKS, J. L., and B. I. HAYMAN, 1953 The analysis of diallel crosses. Maize Gen. Coop. News Let. **27:** 48–54.

MATHER, K., 1949 Biometrical Genetics. London, Methuen & Co.

MATHER, K., and A. VINES, 1953 The inheritance of height and flowering time in a cross of *Nicotiana rustica*. Quantitative Inheritance, H.M.S.O. London, 49–79.

YATES, F., 1947 Analysis of data from all possible reciprocal crosses between a set of parental lines. Heredity **1:** 287–301.

THE THEORY AND ANALYSIS OF DIALLEL CROSSES*

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1. INTRODUCTION

THE diallel cross method of investigating the genetical properties of a group of homozygous lines has recently received much attention. HULL (1945) has considered some aspects of the method. A short summary of a more general approach by JINKS and HAYMAN (1953) and its application to several published sets of maize data has also appeared. In another paper JINKS (1954) has described experiments on inbred lines of *Nicotina rustica*, and has given an account of some of the associated statistics together with a discussion of the results. In this paper we apply a genetic algebra to the theory of the diallel cross, not only to re-establish the formulae of JINKS, but also to investigate more complex genetical systems. We will show how to measure additive and dominance variation, how to describe the relative dominance properties of the parental lines and how to detect non-allelic genic interaction. A worked example illustrates the theory.

The following definitions will be used. A *diallel cross* is the set of all possible matings between several genotypes. The genotypes may be defined as individuals, clones, homozygous lines, etc., and, if there are n of them, there are n^2 mating combinations, counting reciprocals separately. A *diallel table* is an arrangement in a square of n^2 measurements corresponding one-to-one to the mating combinations of a diallel cross, each row and column of the square corresponding to offspring with a common parental genotype. This general definition is necessary because a diallel table need not be restricted to containing measurements on the progeny of a diallel cross, but may be used for later generations obtained by selfing these progeny or backcrossing them to their parents. We shall investigate the diallel cross consisting of the progeny of n selfed homozygous lines and their $n^2 - n$ crosses.

2. A SIMPLE GENETICAL SYSTEM

2.1. Hypotheses. Certain of the hypotheses listed below hold in many genetical systems; the others are useful simplifications and the effects of their failures are discussed in the 4th section. We assume;

(i) Diploid segregation,
(ii) No difference between reciprocal crosses,

* Part of the cost of the accompanying mathematical formulae and tables has been paid by the GALTON AND MENDEL MEMORIAL FUND.

(iii) Independent action of non-allelic genes, and in the diallel cross:
(iv) No multiple allelism
(v) Homozygous parents
(vi) Genes independently distributed between the parents.

2.2. Algebra. Consider a metrical character controlled by k genes, each with two alleles, *A* & *a*, . . . *I* & *i*, ... MATHER (1949) has discussed the case where genes at non-homologous loci influence the character independently and has investigated various mating systems. We consider a method which can be extended beyond sets of independent genes to those in which the genes at non-homologous loci interact. This consists of expressing the (metrical) phenotype as an algebraic function of a suitable representation of the genotype and then, using Mendel's laws to establish the relation between the genotypic representations of parent and progeny, obtaining statistics such as means, variances and covariances by the usual algebraic procedures.

In the notation of MATHER the genotypes *II*, *ii* and *Ii* at the ith locus have phenotypes $c + d_i$, $c - d_i$ and $c + h_i$ respectively where c is constant, $d_i > 0$ and h_i may take either sign. Let us represent the genotype by a variable θ_i which takes the values 1, -1 and 0 respectively, so that the phenotype is the polynomial $c + d_i\theta_i + h_i(1 - \theta_i^2)$. When the genotype controlling the character is the set, $\Theta = (\theta_1, \theta_2, \ldots \theta_k)$, and when genes at non-homologous loci act independently, the phenotype is

$$\sum_{i=1}^{k}\{d_i\theta_i + h_i(1 - \theta_i^2)\}$$

(omitting the constant which does not appear in differences and moments).

Some general results on this representation are:
(i) Since $\theta_i^3 = \theta_i$, $\sum_i\{d_i\theta_i + h_i(1 - \theta_i^2)\}$ is the most general polynomial involving $\theta_1, \theta_2, \ldots \theta_k$ independently, i.e. excluding products like $\theta_i\theta_j$.
(ii) The individual with genotype Θ produces gametes containing *I* and *i* in the frequencies $\frac{1}{2}(1 + \theta_i)$ and $\frac{1}{2}(1 - \theta_i)$.
(iii) The cross $\Theta' \times \Theta''$ (which may be either reciprocal cross) produces progeny *II*, *ii* and *Ii* in the frequencies $\frac{1}{4}(1 + \theta_i')(1 + \theta_i'')$, $\frac{1}{4}(1 - \theta_i')(1 - \theta_i'')$ and $\frac{1}{2}(1 - \theta_i'\theta_i'')$, i.e. these are the probabilities that $\theta_i = 1$, -1 or 0 in the progeny.
(iv) In the progeny of the cross $\Theta' \times \Theta''$ the expectations of θ_i and $1 - \theta_i^2$ are $\frac{1}{2}(\theta_i' + \theta_i'')$ and $\frac{1}{2}(1 - \theta_i'\theta_i'')$. The expected mean phenotype of these progeny is thus $\frac{1}{2}\sum_i\{d_i(\theta_i' + \theta_i'') + h_i(1 - \theta_i'\theta_i'')\}$.

2.3. The statistics of the diallel table. In this section, only genetical variation is considered, and the phenotypes are taken to be exactly

$$\sum_i\{d_i\theta_i + h_i(1 - \theta_i^2)\}.$$

Environmental variation is discussed in 3.1.

The genotypes of the offspring in the n × n diallel cross are determined

by the parental genotypes which are also the genotypes corresponding to the leading diagonal of the diallel table. The parents are assumed to be homozygous with u_i and v_i ($u_i + v_i = 1$) as the frequencies of parents with positive and negative homozygotes at the ith locus. Thus $\theta_i = 1$ in nu_i parents and $\theta_i = -1$ in nv_i parents. The mean of θ_i is $u_i - v_i = w_i$. Also $\theta_i^2 = 1$ and $\text{var}\,\theta_i = 1 - w_i^2 = 4u_iv_i$. Since the genes are assumed distributed independently in the parents $\text{cov}\,(\theta_i,\theta_j) = 0$ ($i \neq j$).

Let the genotypes of the n parents be $\Theta_r = (\theta_{r1},\theta_{r2}, \ldots \theta_{rk})(r = 1, --n)$ so that their phenotypes are $y_r = \sum_i d_i\theta_{ri}$ with mean $m_{L0} = \sum_i d_iw_i$. The progeny of $\Theta_r \times \Theta_s$ (or of $\Theta_s \times \Theta_r$) all have the phenotype $y_{rs} = \frac{1}{2}\sum_i \{d_i(\theta_{ri} + \theta_{si}) + h_i(1 - \theta_{ri}\theta_{si})\} = \frac{1}{2}\sum_i \{(d_i - h_i\theta_{si})\theta_{ri} + d_i\theta_{si} + h_i\}$. The mean of all the progeny of Θ_r (i.e. including Θ_r itself) is

$$\bar{y}_r = \tfrac{1}{2}\sum_i \{(d_i - h_iw_i)\theta_{ri} + d_iw_i + h_i\}$$

and the mean of the whole n^2 progeny is $m_{L1} = \sum_i \{d_iw_i + \frac{1}{2}h_i(1 - w_i^2)\}$. The difference between the mean of the parents and the mean of their n^2 progeny is $m_{L1} - m_{L0} = \frac{1}{2}\sum_i h_i(1 - w_i^2)$.

Consider the set of parents, the rth array (complete row or column) and the set of array means of the diallel table. The variance of the parents,

$$V_{0L0} = \text{var}_r \sum_i d_i\theta_{ri} = \sum_i d_i^2 \,\text{var}_r\, \theta_{ri} = \sum_i d_i^2(1 - w_i^2).$$

The covariance between the parents and their offspring in the rth array,

$$\begin{aligned} W_{01(r)L01} &= \text{cov}\,[\textstyle\sum_i d_i\theta_{si}, \frac{1}{2}\sum_i \{(d_i - h_i\theta_{ri})\theta_{si} + d_i\theta_{ri} + h_i\}] \\ &= \tfrac{1}{2}\textstyle\sum_i d_i(d_i - h_i\theta_{ri})\,\text{var}_s\,\theta_{si} \\ &= \tfrac{1}{2}\textstyle\sum_i d_i(d_i - h_i\theta_{ri})(1 - w_i^2) \end{aligned}$$

The variance of the rth array,

$$\begin{aligned} V_{1(r)L1} &= \tfrac{1}{4}\,\text{var}_s \textstyle\sum_i \{(d_i - h_i\theta_{ri})\theta_{si} + d_i\theta_{ri} + h_i\} \\ &= \tfrac{1}{4}\textstyle\sum(d_i - h_i\theta_{ri})^2(1 - w_i^2). \end{aligned}$$

The covariance between the array means and the rth array,

$$W_{01(r)L1} = \tfrac{1}{4}\textstyle\sum(d_i - h_i\theta_{ri})(d_i - h_iw_i)(1 - w_i^2).$$

The means of the last three statistics are

$$W_{0L01} = \tfrac{1}{2}\textstyle\sum d_i(d_i - h_iw_i)(1 - w_i^2)$$

which is also the covariance between the parents and the means of their offspring,

$$V_{1L1} = \tfrac{1}{4}\textstyle\sum(d_i^2 - 2d_ih_iw_i + h_i^2)(1 - w_i^2),$$

and

$$V_{0L1} = \tfrac{1}{4}\textstyle\sum(d_i - h_iw_i)^2(1 - w_i^2)$$

which is also the variance of the means of the arrays.

Notation. The suffix L refers to the diallel cross mating system and its extension by selfing. The subsequent figure(s), commencing with zero for the parents, refer to the generation(s) under consideration. In variances of individual measurements the preceding figure not in brackets is the same as that following; in variances of means and in covariances the preceding figure(s) refer to the generation(s) of the common parent(s) from which these means are descended. The bracketed figure(s) occur in variances and covariances of arrays and their omission indicates averaging over all arrays. In the L2 generation obtained by selfing the L1 individuals (which are the progeny of the original diallel cross) the variances within progenies are $V_{2(rs)L2}$ and these may be averaged, firstly to give $V_{2(r)L2}$ for each array, and secondly to give V_{2L2}. The extension of the notation over any number of generations of selfing parallels those of MATHER and VINES (1952) for continued selfing or sib-mating from a single cross, except that here we are also interested in statistics from parts of the diallel table. For convenience $W_{0l(r)L01}$ and $V_{1(r)L1}$ will be abbreviated to W_r and V_r.

2.4. Genetical components. The statistics in the previous section (with the omission of the here unimportant $W_{0l(r)L1}$) may be written in a form similar to that used by MATHER (l.c.) as

$$V_{0L0} = D$$
$$W_r = \tfrac{1}{2}D - \tfrac{1}{4}F_r$$
$$W_{0L01} = \tfrac{1}{2}D - \tfrac{1}{4}F$$
$$V_r = \tfrac{1}{4}D - \tfrac{1}{4}F_r + \tfrac{1}{4}H_1$$
$$V_{1L1} = \tfrac{1}{4}D - \tfrac{1}{4}F + \tfrac{1}{4}H_1$$
$$V_{0L1} = \tfrac{1}{4}D - \tfrac{1}{4}F + \tfrac{1}{4}H_1 - \tfrac{1}{4}H_2$$

and
$$m_{L1} - m_{L0} = \tfrac{1}{2}h$$

where
$$D = \Sigma d_i^2(1 - w_i^2)$$
$$F_r = 2\Sigma d_i h_i \theta_{ri}(1 - w_i^2)$$
$$F = 2\Sigma d_i h_i w_i(1 - w_i^2)$$
$$H_1 = \Sigma h_i^2(1 - w_i^2)$$
$$H_2 = \Sigma h_i^2(1 - w_i^2)^2$$

and
$$h = \Sigma h_i(1 - w_i^2)$$

Those equations independent of r may be solved directly for D, F, H_1, H_2 and h. $D - F + H_1 - H_2 (4V_{0L1})$ and H_2 are MATHER's (l.c.p.75) random mating D and H. When $w_i = 0$, D and H_1 (or H_2) become MATHER's (l.c.P.56) selfing and sib-mating D and H.

2.5. Distribution of alleles. $H_1 - H_2 = \Sigma h_i^2 w_i^2(1 - w_i^2) \geqslant 0$. Now $w_i^2 \not\equiv 1$. Hence, if some loci exhibit dominance ($H_1 \neq 0$), the vanishing of $H_1 - H_2$ means that $w_i = 0$ ($u_i = v_i$) at these loci, while $H_1 > H_2$ means that $u_i \neq v_i$, i.e. the positive and negative alleles at these loci are not in equal proportions in the parents. Since $H_1 - H_2$ depends on the square of w_i it is not possible to decide in the latter case whether the positive or negative alleles are in excess. Now $w_i^2(1 - w_i^2)$ vanishes at $w_i = 0$, increases slowly with w_i^2 to a maximum at $w_i^2 = \tfrac{1}{2}$(u_i:v_i = 0·85:0·15 or 0·15:0·85) and then decreases to zero at $w_i^2 = 1$. Hence $H_1 - H_2$ fails to detect weak

asymmetry in general and extreme asymmetry in large diallel crosses. A test of significance of $H_2 - H_1$ is referred to in 3.3. An estimator of the mean value of u_iv_i at loci exhibiting dominance is $H_2/4H_1$. This is, however, biassed towards the larger values of u_iv_i.

2.6. Dominance. In qualitative definitions of dominance the heterozygote of the gene is taken to have the same phenotype as one of the homozygotes—the dominant homozygote—so that parents which contain the greatest number of dominant homozygotes of the genes controlling the character in question produce offspring with the least variation among themselves and with the least covariation with their other and more recessive parents in that character.

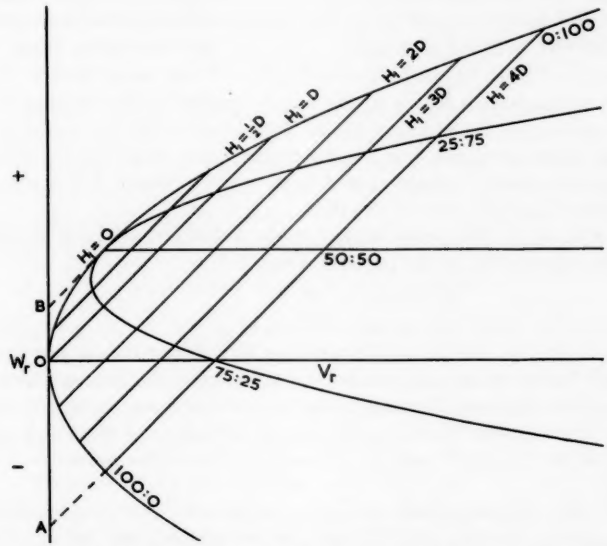

FIGURE 1.—Diallel cross dominance relationships in terms of V_r, the variance of all the offspring of the rth parent, and W_r, the covariance between these offspring and their non-recurrent parents, environmental variation being neglected.

For a diallel cross with a certain value of H_1/D, the points (V_r, W_r) are distributed along a corresponding straight line of unit slope inside the limiting parabola, $W_r^2 = V_r V_{0L0}$. This is one of the full sloping lines in the diagram. If the continued line cuts the W_r-axis in A, and if the parallel tangent to the parabola cuts it in B, then the line is determined by $AB/OB = H_1/D$. The line marked A in the diagram corresponds to a diallel cross with $H_1/D = 4$.

The position of (V_r, W_r) on the line reveals the relative proportions of dominant and recessive genes in the rth parent. For any diallel cross, the point corresponding to a parent containing p% dominants and q% recessives lies on the curve labelled p:q. Completely recessive parents correspond to points at the upper ends of the sloping lines on the part of the limiting parabola labelled 0:100, and completely dominant parents to points at the lower ends on the part labelled 100:0.

In an experiment with no dominance all the points coincide at $(\frac{1}{4}D, \frac{1}{2}D)$ ($H_1 = 0$ in the diagram).

Quantitatively we may consider any degree of dominance (measured by $|h_i|/d_i$ at the ith locus), the dominant homozygote deviating from the mid-homozygote in the same direction as the heterozygote so that $h_i\theta_i = |h_i|$. Similarly $h_i\theta_i = -|h_i|$ for the recessive homozygote. ($|h_i|$ means the positive value of h_i.)

In the diallel cross an overall measure of dominance is provided by $(H_1/D)^{\frac{1}{2}}$, the square root of the ratio of weighted means of h_i^2 and d_i^2. This ratio may be obtained from the graph of W_r against V_r. From 2.4, $W_r - V_r = \frac{1}{4}(D - H_1) = W_{0L01} - V_{1L1}$, so that the points (V_r,W_r) lie on a straight line of unit slope through their mean point (V_{1L1},W_{0L01}). The statistical inequality $W_r^2 \leqslant V_rV_{0L0}$ means that points can only lie on that part of the line inside the parabola $W_r^2 = V_rV_{0L0}$. Let the line cut the OW axis in A and let the parallel tangent to this limiting parabola cut the same axis in B. Then $AB/OB = H_1/D$ and this is identical with the value given by the equations in 2.4. In figure 1 the line $H_1/D = 4$ has been labelled A. When there is no dominance ($H_1 = 0$) the line is tangent to the limiting parabola and all the points (V_r,W_r) coincide at the point of contact, $(\frac{1}{4}D,\frac{1}{2}D)$. With complete dominance the line passes through the origin (0,0) while with partial dominance it lies above and with overdominance below the origin. In the latter case W_r may be negative.

With the aid of this graph we can make a detailed study of the relative dominance properties of the parents. From 2.4,

$$W_r = \tfrac{1}{2}D - \tfrac{1}{4}F_r$$

and

$$V_r = \tfrac{1}{4}D - \tfrac{1}{4}F_r + \tfrac{1}{4}H_1$$

where $F_r = 2\Sigma d_ih_i\theta_{ri}(1 - w_i^2)$. Since $h_i\theta_{ri}$ is positive for a dominant homozygote and negative for a recessive the greater values of F_r correspond to the more dominant parents and the lesser values to recessive parents. Thus, in the (V_r,W_r) graph, points with lower values of V_r and W_r correspond to dominant parents and points with higher values to recessive parents.

If we define the completely dominant parent to be the (possibly fictitious) parent carrying the dominant homozygotes of all the genes (which may have either positive or negative effects) and the completely recessive parent that carrying all the recessive homozygotes then, for the complete dominant,

$$V_D = \tfrac{1}{4}\Sigma(d_i - |h_i|)^2(1 - w_i^2)$$

and for the complete recessive

$$V_R = \tfrac{1}{4}\Sigma(d_i + |h_i|)^2(1 - w_i^2).$$

Now, unless the degree of dominance, $|h_i|/d_i$, of every gene is the same, (V_D,W_D) and (V_R,W_R) lie just inside the parabola. However, assuming that the points where the straight line cuts the parabola correspond to the completely dominant and recessive parents, it is found that

$$(V_D,W_D) = (V_{0L0}x_1^2,V_{0L0}x_1)$$

and

$$(V_R,W_R) = (V_{0L0}x_2^2,V_{0L0}x_2)$$

where x_1 and x_2 are the roots of $V_{0L0}x^2 - V_{0L0}x + W_{0L01} - V_{1L1} = 0$.

Suppose that the parent Θ_r contains k_{rD} dominant genes and k_{rR} recessive genes. Then, with certain restrictions about equality of gene effects,

$\frac{k_{rD}}{k_{rR}} = \frac{V_R - V_r}{V_r - V_D}$ = the ratio of the lengths of the two segments into which the point (V_r,W_r) divides the chord joining (V_R,W_R) to (V_D,W_D). (See fig. 1). The ratio of the total numbers of dominant to recessive genes in all the parents is

$$\frac{k_D}{k_R} = \frac{V_R - V_{1L1}}{V_{1L1} - V_D} = \frac{(4DH_1)^{\frac{1}{2}} + F}{(4DH_1)^{\frac{1}{2}} - F}.$$

2.7. Dominance by regression methods. With no dominance the regression of progeny with one parent in common on their non-recurrent parents is a straight line of slope $\frac{1}{2}$. When dominance is present consider the measurements, y_{rs}, of the progeny of Θ_r—the rth array—and the parental measurements, $y_s (s = 1, \ldots n)$. We have $y_{rs} - \frac{1}{2}y_s = \frac{1}{2}\sum_i \{d_i\theta_{ri} + h_i(1 - \theta_{ri}\theta_{si})\}$, which is not now independent of s, and in fact a linear relationship no longer exists between y_{rs} and y_s for given r. A best fitting regression line may, of course, be found for the offspring of each parent and its slope, W_r/V_{0L0}, will vary from parent to parent. HULL (1945, 1952) uses this variation in slope to detect and measure dominance.

His method is to fit the regression surface

$$y_{rs} = c + \tfrac{1}{2}b_1(y_r + y_s) - b_2y_ry_s$$

to the diallel table. b_2 is also the regression of regression slopes onto the corresponding parents and its existence indicates the presence of dominance. HULL's estimator of the degree of dominance reduces to $\{(1 - b_1)^2 + 4cb_2\}^{\frac{1}{2}}$. Fitting his regression surface to our more general genetical model we find that this estimator becomes

$$\begin{aligned}&\{(V_{0L0} - 2W_{0L01})^2 + 4\mathrm{Cov}(W_r,y_r)(m_{L0} - m_{L1})\}^{\frac{1}{2}}/V_{0L0}\\ &= \{(\Sigma d_ih_iw_i(1 - w_i^2))^2 + \Sigma d_i^2h_i(1 - w_i^2)^2 \,.\, \Sigma h_i(1 - w_i^2)\}^{\frac{1}{2}}/\Sigma d_i^2(1 - w_i^2)\\ &= (\Sigma d_i^2h_i \,.\, \Sigma h_i)^{\frac{1}{2}}/\Sigma d_i^2, \text{ in the special case when all } w_i = 0.\end{aligned}$$

Since HULL, unlike us, omits the diagonal of the diallel table in computing parent-offspring covariances these are not exact representations of his estimator but they suffice to reveal the main differences from our estimator, $(H_1/D)^{\frac{1}{2}} = (\Sigma h_i^2/\Sigma d_i^2)^{\frac{1}{2}}$ when all $w_i = 0$.

Clearly HULL's estimator measures mean dominance and not mean square dominance and must underestimate the average degree of dominance in any case but that of unidirectional dominance (for which it was admittedly designed). The regression coefficient, b_2, which provides the test of significance of dominance suffers from the same disadvantage. Furthermore, the third degree statistic, $\mathrm{Cov}(W_r,y_r)$, is a greater source of sampling error than the second degree statistics used in our estimator. It is interesting to note that, perhaps contrary to expectation, our more general theory has enabled us to obtain the simpler and more reliable estimator of the degree of dominance.

2.8. Number of genes. An estimate of the summed value of h_i is (2.4) $2(m_{L1} - m_{L0}) = h = \Sigma h_i(1 - w_i^2)$. In general this underestimates mean dominance because positive and negative values of h_i cancel out, but its sign does show whether positive or negative dominants are in the majority, or which exhibit the greatest degree of dominance.

Let k_+ and k_- be the number of groups of genes distributed independently in the parents for which the dominance is respectively positive and negative. Then $\frac{h^2}{H_2} = \frac{(k_+ - k_-)^2}{(k_+ + k_-)}$ again with certain restrictions about equality of gene effects. Now, if either k_+ or k_- is zero, i.e. all dominants have the same sign, this ratio estimates the number of groups which control the character and exhibit dominance to some degree. Usually, however, it underestimates this number, and it provides no information about groups of genes exhibiting little or no dominance. These groups of genes are not to be confused with MATHER's (l.c.) effective factors.

It is important to distinguish the different discussions of the gene distribution in the previous sub-sections. The proportions in all the parents of positive and negative homozygotes at each locus which exhibits dominance are considered in 2.5; 2.6 gives the relative proportions of dominant and recessive homozygotes in each parent; here a lower bound is found to the number of genes exhibiting dominance in the parents.

2.9. Dominance and size. The sign of h (2.8) gives the mean direction of dominance. A measure of association between the signs of dominant genes is the correlation between parental size and parental order of dominance. The parental measurement, y_r, is closely correlated with the number of positive homozygotes in the parent while $(W_r + V_r)$ bears the same relation to the number of recessive homozygotes (3.2). When the correlation, ρ, between y_r and $(W_r + V_r)$ is nearly one the recessive genes must be mostly positive; when ρ is minus one the dominant genes are positive; when ρ is small equal proportions of the dominant genes are positive and negative.

When ρ^2 is nearly unity, the regression of y_r on $(W_r + V_r)$ exists, and the substitution of $(W_R + V_R)$ and $(W_D + V_D)$ in the regression equation predicts the measurements of the completely dominant and recessive parents. These must also be predictions of the possible limits of selection from amongst the genes exhibiting dominance, but, as before, we have no information about possible limits of selection from amongst the other genes.

3. COMPONENTS OF VARIATION

3.1. Environmental variation. Interaction between environmental fluctuations and the genotypes in a diallel cross is revealed by heterogeneity of the variances within (or between duplicate) parental and F_1 families. Such heterogeneity may be handled in at least three cases.

(i). When the sole source of heterogeneity is a difference between parental and F_1 variances the environmental variances of y_r and y_{rs} $(r \neq s)$ may be denoted by E and $\frac{1}{2}E'$ respectively. E is estimated from differences between

duplicate plots, E′ is estimated from the same source or from reciprocal differences, and the factor of $\frac{1}{2}$ compensates for the replacement of each pair of measurements of reciprocals by their common mean, which is done before evaluating statistics from the diallel table. With the environmental expectations included the equations of 2.4 become

$$\begin{aligned}
V_{0L0} &= D + E \\
W_r &= \tfrac{1}{2}D - \tfrac{1}{4}F_r + E/n \\
W_{0L01} &= \tfrac{1}{2}D - \tfrac{1}{4}F + E/n \\
V_r &= \tfrac{1}{4}D - \tfrac{1}{4}F_r + \tfrac{1}{4}H_1 + (E + \tfrac{1}{2}(n-1)E')/n \\
V_{1L1} &= \tfrac{1}{4}D - \tfrac{1}{4}F + \tfrac{1}{4}H_1 + (E + \tfrac{1}{2}(n-1)E')/n \\
V_{0L1} &= \tfrac{1}{4}D - \tfrac{1}{4}F + \tfrac{1}{4}H_1 - \tfrac{1}{4}H_2 + (E + \tfrac{1}{2}(n-2)E')/n^2 \\
(m_{L1} - m_{L0})^2 &= \tfrac{1}{4}h^2 + (n-1)((n-1)E + E')/n^3.
\end{aligned}$$

Even W_r and W_{0L01} have environmental expectations because each array has a term in common with the parental array.

(ii). When each F_1 variance can be expressed as the sum of two components corresponding to its parents those of the above equations which are independent of r hold with $E = E' =$ overall mean family variance.

(iii). When a trend exists between all the L_1 family means and variances the genotype-environment interaction may be removed by rescaling. WRIGHT (1952) describes the standard method.

When the influence of the environment is independent of genotype all the above equations hold with $E' = E$. We use them in this form in the next sub-section.

3.2. Accuracy of the components. Those of the equations in 3.1(i) which are independent of r, together with an estimate of E, furnish an exact solution for D, F, H_1, H_2 and h^2, but no estimate of their accuracy. However, we have observed (2.6) that there are n estimates, $W_r - V_r$, of $\frac{1}{4}(D - H_1)$, and the sampling variation in these may be used to provide approximate standard errors of the genetical and environmental components. In replicated experiments, block differences supply a further estimate of error.

In obtaining the least squares solution of the above equations we omit W_{0L01} and V_{1L1} as superfluous (because of W_r and V_r) and weight with a factor $n^{\frac{1}{2}}$ the equations for V_{0L1} and $(m_{L1} - m_{L0})^2$ and the estimate of E since, unlike the others, these three statistics depend on all the measurements of the diallel table. The solution is

$$\begin{aligned}
\hat{D} &= V_{0L0} - \hat{E} \\
\hat{F} &= 2V_{0L0} - 4W_{0L01} - 2(n-2)\hat{E}/n \\
\hat{H}_1 &= V_{0L0} - 4W_{0L01} + 4V_{1L1} - (3n-2)\hat{E}/n \\
\hat{H}_2 &= 4V_{1L1} - 4V_{0L1} - 2\hat{E} \\
\hat{h}^2 &= 4(m_{L1} - m_{L0})^2 - 4(n-1)\hat{E}/n^2 \\
\hat{F}_r &= 2(V_{0L0} - W_{0L01} + V_{1L1} - W_r - V_r) - 2(n-2)\hat{E}/n
\end{aligned}$$

where we have used W_{0L01} and V_{1L1} for the means of W_r and V_r. The estimates of D, F, H_1, H_2 and h^2 are the same as in the exact solution. Only the

TABLE 1

Covariance Matrix

	D	F	H_1	H_2	h^2	E
D	$n^5 + n^4$	$2n^5 + 2n^4 - 4n^3$	$n^5 + 3n^4 - 2n^3$	$2n^4$	$4n^3 - 4n^2$	$-n^4$
F	$2n^5 + 2n^4 - 4n^3$	$4n^5 + 20n^4 - 16n^3 + 16n^2$	$2n^5 + 22n^4 - 16n^3 + 8n^2$	$4n^4 - 8n^3$	$8n^3 - 24n^2 + 16n$	$-2n^4 + 4n^3$
H_1	$n^5 + 3n^4 - 2n^3$	$2n^5 + 20n^4 - 16n^3 + 8n^2$	$n^5 + 41n^4 - 12n^3 + 4n^2$	$22n^4 - 4n^3$	$12n^3 - 20n^2 + 8n$	$-3n^4 + 2n^3$
H_2	$2n^4$	$4n^4 - 8n^3$	$22n^4 - 4n^3$	$36n^4$	$8n^3 - 8n^2$	$-2n^4$
h^2	$4n^3 - 4n^2$	$8n^3 - 24n^2 + 16n$	$12n^3 - 20n^2 + 8n$	$8n^3 - 8n^2$	$16n^4 + 16n^2 - 32n + 16$	$-4n^3 + 4n^2$
E	$-n^4$	$-2n^4 + 4n^2$	$-3n^4 + 2n^3$	$-2n^4$	$-4n^3 + 4n^2$	n^4

with common multiplier s^2/n^5

Also $\mathrm{Var}F_r = 4(3n^3 + 3n^2 - 4n + 4)s^2/n^3$

$\mathrm{Cov}(F_r, F_s) = 4(n^3 + 3n^2 - 4n + 4)s^2/n^3 \quad (r \neq s)$

$\mathrm{Var}(D - H_1) = 4(9n^2 - 2n + 1)s^2/n^3$

estimate of F_r is new, and it shows that $(W_r + V_r)$, and not just V_r or W_r, provides the better measure of dominance order (2.6).

The expected values of the statistics, derived from the estimates of the components, are identical with the observed values for V_{0L0}, V_{0L1}, $(m_{L1} - m_{L0})^2$ and E, but

$\hat{W}_r = \frac{1}{2}(W_{0L01} - V_{1L1} + W_r + V_r)$
and $\hat{V}_r = \frac{1}{2}(-W_{0L01} + V_{1L1} + W_r + V_r)$ so that the residual sum of squares $= \frac{1}{2}\{\Sigma(W_r - V_r)^2 - n(W_{0L01} - V_{1L1})^2\}$ with $n - 1$ degrees of freedom. The mean square, $s^2 = \frac{1}{2}\mathrm{Var}(W_r - V_r)$. The covariance matrix of $\hat{D}$, $\hat{F}_r$, $\hat{H}_1$, $\hat{H}_2$, $\hat{h}^2$ and $\hat{E}$ is the inverse of the matrix of the coefficients of these components in the least squares equations and, as the important components are D, F, H_1, H_2, h^2 and E, the covariance matrix may be contracted to refer only to these quantities (see table 1).

The sampling correlation between $\hat{D}$ and $\hat{H}_1$ is positive so that diallel crossing shares with repeated backcrossing from the F_1 of a single cross the advantage over continued selfing or sib-mating of enabling $D - H_1$ to be estimated with the greater accuracy. In fact, if we solely desire to test the deviation of $\hat{D} - \hat{H}_1$ from zero we may use the direct estimate, $\mathrm{Var}(\hat{D} - \hat{H}_1) = 16\mathrm{Var}(W_r - V_r)/n$. Combined with the test of the significance of H_2 (3.3), this quick test at once classifies the experiment into one of four categories as exhibiting no dominance, partial dominance, complete dominance or overdominance.

3.3. Analysis of variance. In another paper HAYMAN (1954) has constructed an analysis of variance of the diallel table to test additive and dominance variation in the multiple allele case. Such an analysis provides statistically sound tests of significance of some of the components discussed here, viz., $(D - F + H_1 - H_2)$ (i.e. V_{0L1}), H_2, $(H_1 - H_2)$ and h^2 as well as differences between reciprocal crosses. See table 3 of that paper and 6.3 in this paper. The error mean square from this analysis of variance may be used as an estimate of E.

4. GENERAL GENETICAL SYSTEMS

4.1. Testing the hypotheses. Before the theory of the previous sections can be applied to a diallel table it is necessary to show that the corresponding diallel cross conforms to the hypotheses postulated in 2.1. A consequence of those hypotheses was that $W_r - V_r$ was constant, i.e. independent of r (2.6). We therefore expect that failures of the hypotheses may upset this constancy, and this is borne out in the investigations below. Heterogeneity of $W_r - V_r$ is thus a good indication of such failures. Homogeneity of $W_r - V_r$, while always implied by the validity of the hypotheses, may also be attained in certain cases of balanced failure. Two tests for heterogeneity of $W_r - V_r$ are available.

(i). When the experiment is replicated the variance of $W_r - V_r$ may be analysed for line and block differences. A significant line effect indicates fail-

ure of the hypotheses. The main statistical fault in this test is that the n values of $W_r - V_r$ are correlated. This paragraph, with 3.2, reveals the importance of the variance of $W_r - V_r$ in the analysis of diallel crosses.

(ii). A test which is useful when the experiment is not replicated depends on the (V_r,W_r) graph. This is not a line of unit slope if $W_r - V_r$ varies. To provide a test which gives equal weight to both W_r and V_r the axes of the graph are rotated through 45° so that the coordinates of points become proportional to $W_r + V_r$ and $W_r - V_r$. The t testing the significance of regression in the new axes is given by

$$t^2 = \frac{n-2}{4} \cdot \frac{(\mathrm{Var}V_r - \mathrm{Var}W_r)^2}{\mathrm{Var}V_r\mathrm{Var}W_r - \mathrm{Cov}^2(V_r,W_r)} \text{ with } n-2 \text{ degrees of freedom.}$$

Significance indicates failure of the hypotheses. The weakness of this test is that it only detects variation in $W_r - V_r$ which is correlated with the dominance order of the parents. Variation which merely increases the scatter of points about the regression line without altering its slope can only be detected by the first test.

Table 2 lists, for both tests, the probabilities of the hypotheses holding for three characters from a diallel cross of *Nicotiana rustica* varieties which was repeated for three years. Asterisks indicate failure of the hypotheses. Evidently experiments should be replicated to ensure detection of such failures.

TABLE 2

Significance tests applied to the hypotheses of 2.1 *for height, flowering time, and leaf length by the two tests described. Asterisks indicate failure of the hypothesis.*

Character		Test (i)	Test (ii)
Height	1951*	.01–.001	.20–.10
	1952*	.01–.001	.10–.05
	1953*	.01–.001	.10–.05
Flowering time	1951	.05–.01	> .90
	1952*	.01–.001	.01–.001
	1953	.05–.01	.70–.60
Leaf length	1951	.10–.05	.80–.70
	1952	> .20	.80–.70

When failure of the hypotheses has been demonstrated the simple theory of section 2 is no longer applicable; a more complex genetical system must be postulated and new parameters introduced to represent it. In the absence of data from later generations the components will outnumber the statistics so that a solution for them cannot be found. However, it is still possible to draw the (V_r,W_r) graph and to make estimates of D, F, H_1, H_2 and h^2 from the formulae in 3.2. We will now discuss what genetical interpretations can be ascribed to such components and what forms the graph may take under failure of the various hypotheses. Let OW be the vertical axis and OV the horizontal axis.

4.2. Reciprocal differences. When reciprocal differences exist, two values of each statistic are obtained from the table, one by using the columns, and the other by using the rows, as arrays. This ambiguity may be removed by replacing all entries in the table by their mean reciprocals. If the differences were independent of genotype then estimates of the genetical components are now correct as long as E contains the variation between reciprocals. If the differences depended on genotype then the genetical components are only averages in some way over maternal effects. 3.3 refers to a test for reciprocal differences.

4.3. Residual heterozygosity in the parents. A full treatment of diallel crosses between partially inbred material will require a separate paper and only the pertinent results are mentioned here. Firstly, the (V_r,W_r) graph is a scatter about a line of unit slope, points above the line corresponding to heterozygous parents and points below the line to inbred parents. Secondly, the formulae of 3.2 produce underestimates of H_1/D and $H_2/4H_1$ and an overestimate of F so that the degree of dominance is underestimated, asymmetry of the gene distribution exaggerated and the proportion of dominants overestimated.

4.4. Correlated gene distributions. When the genes at different loci are uncorrelated $\underset{r}{\mathrm{Cov}}(\theta_{ri},\theta_{rj}) = 0 (i \neq j)$ but in the general case we must take $\underset{r}{\mathrm{Cov}}(\theta_{ri},\theta_{rj}) = c_{ij}$. Then, neglecting E, the formulae of 3.2 give

$$\hat{D} = \sum_i d_i^2(1 - w_i^2) + \sum_{i \neq j} d_i d_j c_{ij}$$
$$\hat{F} = 2\sum_i d_i h_i w_i(1 - w_i^2) + 2\sum_{i \neq j} d_i h_j w_j c_{ij}$$
$$\hat{H}_1 = \sum_i h_i^2(1 - w_i^2) + \sum_{i \neq j} h_i h_j c_{ij}(w_i w_j + c_{ij})$$
$$\hat{H}_2 = \sum_i h_i^2(1 - w_i^2)^2 + \sum_{i \neq j} h_i h_j c_{ij}^2$$
$$\hat{h} = \sum_i h_i(1 - w_i^2).$$

Taking all $w_i = 0$ for simplicity we get

$$\hat{D} = \sum_i d_i^2 + \sum_{i \neq j} d_i d_j c_{ij}$$
$$\hat{F} = 0$$
$$\hat{H}_1 = \sum_i h_i^2 + \sum_{i \neq j} h_i h_j c_{ij}^2 = \hat{H}_2$$
$$\hat{h} = \sum_i h_i$$

Since $\hat{F} = 0$ and $\hat{H}_1 = \hat{H}_2$ the measures of gene frequency are unaffected by the correlation. $\hat{H}_1$ (or $\hat{H}_2$) is greater than $\sum h_i^2$ when the dominance is unidirectional, and slightly less if the h_i are randomly distributed in sign and magnitude. The estimate of gene number, $\hat{h}^2/\hat{H}_2$, is therefore usually depressed by the occurrence of correlation. $\hat{D}$ $(= V_{0L0})$ is zero when each parent has the same phenotype, and this occurs if each parent contains a suitable combination of positive and negative genes (dispersion). When genes of like effect are together in each parent (association) most $c_{ij} > 0$ and $\hat{D}$ is greater than $\sum d_i^2$. Thus the measure of degree of dominance, $\hat{H}_1/\hat{D}$, may be either increased or decreased by the combined effects of correlation on $\hat{H}_1$ and $\hat{D}$ but the particular combination of dispersion and unidirectional dominance causes serious inflation and may easily turn partial dominance into apparent overdominance.

The effect on the (V_r,W_r) graph is interesting. When the c_{ij} are small the values of V_r still depict the order of dominance. Further

$$W_r - V_r = \tfrac{1}{4}\Sigma_i(d_i^2 - h_i^2)(1 - w_i^2) + \tfrac{1}{4}\Sigma_{i\neq j}(d_id_j - h_ih_j\theta_{ri}\theta_{rj})c_{ij}.$$

The completely dominant and recessive parents correspond to $W_D - V_D = W_R - V_R = \tfrac{1}{4}\Sigma(d_i^2 - h_i^2)(1 - w_i^2) + \tfrac{1}{4}\Sigma(d_id_j - |h_ih_j|)c_{ij}$. This is on the average the minimum value of $W_r - V_r$ for association and the maximum value for dispersion. Parents with intermediate proportions of dominants and recessives correspond to the maximum and minimum values respectively. Hence with association the (V_r,W_r) curve is convex upwards and with dispersion convex downwards.

4.5. Non-allelic gene interaction. Like 4.3, this heading provides scope for a separate paper. However, we can mention here that, now that a perfectly general representation of gene effect has been developed (HAYMAN and MATHER 1955), genic interaction presents no insuperable problems and we can give some of the pertinent results. A complementary type of interaction distorts the (V_r,W_r) graph, inflates $\hat{H}_1/\hat{D}$, depresses $\hat{h}^2/\hat{H}_2$ but has little effect on the estimators of gene frequency. A duplicate type of interaction depresses $\hat{h}^2/\hat{H}_2$, increases the apparent proportion of dominants but leaves $\hat{H}_1/\hat{D}$ and $\hat{H}_2/4\hat{H}_1$ and the (V_r,W_r) graph almost unaltered.

4.6. Scaling. Interaction between genes at non-homologous loci in a single cross may often be eliminated by a suitable change of the scale of measurement, but when interaction has been shown to be present in a diallel cross it may well be impossible to find one scale on which every individual cross exhibits no interaction. This can, nevertheless, be accomplished when a trend exists between $W_r - V_r$ on the one hand and both the corresponding parental and array mean values, y_r and $\bar{y}_r$, on the other hand. The method is given by WRIGHT (l.c.).

4.7. Multiple allelism. In the absence of segregation this is equivalent to polygenic biallelism exhibiting genic interaction and distributional correlation as illustrated here. A gene with four alleles forms four homozygotes and is thus equivalent to two genes with two alleles each. There are 9 independent comparisons between ten genotypes, whether derived from the four multiple alleles of one gene, or from two biallelic genes. However, whereas in the first case these comparisons correspond to additive and dominance effects, in the second case only 4 of the comparisons have this property, the others corresponding one to position effect and 4 to genic interaction. Any set of 2^p multiple alleles is equivalent in a like manner to p pairs of alleles. A set of q ($\neq 2^p$) multiple alleles requires p pairs of alleles to represent it, where $2^{p-1} < q < 2^p$, but there must also be some genic correlation to reduce the number of homozygotes to q. Evidently the effects of multiple allelism can be extremely complicated.

4.8. When there is no true dominance (all $h_i = 0$) the extended discussion of the previous five sub-sections may be reduced to the simple statement that any failure of the hypotheses which can be detected in the (V_r,W_r) graph causes the components estimated by the formulae of 3.2 to

exhibit spurious dominance. This follows directly from the concept of the limiting parabola in the (V_r,W_r) diagram. When there is no dominance all the points should coincide at $(\frac{1}{4}D,\frac{1}{2}D)$ on the limiting parabola. Any failure of the hypotheses which scatters the points (V_r,W_r) causes their mean (V_{1L1},W_{0L01}) to lie inside and not on the limiting parabola. Therefore, from 2.6, $\hat{H}_1 > 0$.

4.9. Analysis when the hypotheses fail. The approach to the problem of a diallel table exhibiting genic interaction (or indeed any failure of the hypotheses) has been to show how the (V_r,W_r) graph is affected. This is all that can be done with data from one generation because rescaling (4.6) is not possible if the scale has already been fixed to minimise genotype-environment interaction (3.1). Certainly no exact analysis can be undertaken while the genetical system fails to satisfy the hypotheses of section 2.

The way to further progress is to find some sub-table of the diallel table which does satisfy all the hypotheses and from which valid conclusions may be drawn as to the degree of dominance, asymmetry of gene distribution, etc., in the corresponding sub-group of non-interacting lines. Interacting lines usually have extreme values of $W_r - V_r$, i.e. lie well off the line of unit slope through (V_{1L1},W_{0L01}). A surer method of discovering which line to eliminate is to remove the measurements on the offspring of each line in turn and to test for heterogeneity of $W_r - V_r$ in each of the resulting $(n - 1) \times (n - 1)$ diallel tables. If any of these heterogeneities is not significant then, as far as this test is concerned, the corresponding sub-table satisfies the hypotheses and the lines remaining in it may be analysed as in sections 2 and 3.

If the interaction is still significant whichever line is removed, the next step is to remove every possible pair of lines in turn and test the remaining sub-tables for heterogeneity of $W_r - V_r$, then, if necessary, all triples of lines, etc., until a diallel table satisfying the hypotheses is obtained. In practice, if the removal of no single line can eliminate the interaction, it is usually sufficient, and far less laborious, instead of removing every possible pair of lines, to remove that line whose omission minimises the heterogeneity and then to remove each of the remaining lines in turn.

When only two values of $W_r - V_r$ (for r = s and t) show failure of the hypotheses by deviating markedly from the common value of the others, the cause may be interaction in the single cross $\Theta_s \times \Theta_t$. This is the case when it is possible to adjust the value of this phenotype to make $W_s - V_s$ and $W_t - V_t$ revert to the common value of the other $W_r - V_r$. Theoretically this should be done by minimising the heterogeneity of $W_r - V_r$ but in practice a missing plot fit based on the analysis of variance of the diallel table (3.3) seems to be good enough and is simpler. If y_{ts} and y_{st} are fitted values then

$$(n - 2)(n - 3)(y_{st} + y_{ts}) = (n - 1)(y_{s\cdot} + y_{\cdot s} + y_{t\cdot} + y_{\cdot t} - 2y_s - 2y_t) - 2y_{\cdot\cdot} + 2y_{\cdot}$$

and $(n - 2)(y_{st} - y_{ts}) = y_{s\cdot} - y_{\cdot s} - y_{t\cdot} + y_{\cdot t}$ where the sums $y_{s\cdot}$, etc.,

exclude the missing values. If this adjustment eliminates the interaction the whole table may be analysed without having to omit all the progeny either of Θ_s or of Θ_t.

This procedure of selecting certain lines which conform to the hypotheses is open to the criticism that some sub-group of lines is likely to do so merely through sampling variation. Replication of the experiment in time and space is necessary to validate such selection.

5. SUMMARY OF THE METHOD OF ANALYSIS

(i). Test the variances within families of parents and F_1's for genotype-environment interaction. This should lie within one of the categories in 3.1 for further analysis to be possible.

(ii). Form the diallel table of reciprocal means, compute V_r and W_r ($r = 1, \ldots n$) and test $W_r - V_r$ for heterogeneity (4.1). If this is significant, plot $W_r - V_r$ against $\bar{y}_r$ to decide if rescaling would be useful (4.6) and, if this fails, determine the interacting lines or crosses by inspection or otherwise and remove or adjust them (4.9).

(iii). When a diallel table with uniform $W_r - V_r$ has been obtained analyse its variance to find the significance of some of the genetical components of variation and to provide the estimate of E (3.3).

(iv). From V_{0L0}, W_{0L01}, V_{1L1}, V_{0L1}, $(m_{L1} - m_{L0})^2$ and the estimate of E compute $\hat{D}$, $\hat{F}$, $\hat{H}_1$, $\hat{H}_2$ and $\hat{h}^2$ and find their standard errors (3.2).

(v). Evaluate and interpret $\frac{\hat{H}_1}{\hat{D}}$ (2.6), $\frac{\hat{H}_2}{4\hat{H}_1}$ (2.5), $\frac{(4\hat{D}\hat{H}_1)^{\frac{1}{2}} + \hat{F}}{(4\hat{D}\hat{H}_1)^{\frac{1}{2}} - \hat{F}}$ (2.6), and $\frac{\hat{h}^2}{\hat{H}_2}$ (2.8) when the relevant components are significant.

(vi). Note the order of dominance of the parents (3.2). Find the correlation between $W_r + V_r$ and y_r and, if it is significant, predict the limits of selection (2.9).

A quicker and more superficial analysis would omit the analysis of variance of the diallel table and estimate E from differences between reciprocals.

6. NUMERICAL EXAMPLE

6.1. The data used to illustrate the analysis of a diallel table were kindly supplied by Dr. Jinks. They are the flowering times, in days from a date in 1952, of *Nicotiana rustica* plants from a diallel cross of eight inbred varieties. These plants were grown in two blocks each containing 64 plots; each cross or self was represented by 10 progeny, grown in two plots of 5, with one plot in each block. Table 3 contains the mean flowering times per plot of the progeny. The variances within plots do vary significantly, but in the manner described in 3.1(ii) so that it is legitimate to use an average value for E.

6.2. Table 3 also contains the variances, V_r, of arrays and the covariances, W_r, between the arrays and the parental array, calculated for each block from the diallel table of mean reciprocals. $W_r - V_r$ is reasonably constant except for $r = 1$ and 3; the table shows exceptional and consistent heterosis

TABLE 3

Mean flowering time per plot of the progeny of a diallel cross of eight varieties.

Block I

	♀ 1	2	3	4	5	6	7	8	W_r	V_r	$W_r - V_r$
1	22.8	14.4	27.2	17.2	18.3	16.2	18.6	16.4	18.29	24.46	−6.18
2	15.4	17.2	14.8	18.6	15.2	17.0	14.4	10.8	7.44	5.55	1.89
3	31.8	21.0	24.8	24.6	19.2	29.8	12.8	13.0	23.17	30.59	−7.42
4	16.2	11.4	16.8	18.4	12.4	16.8	12.6	9.6	10.37	7.48	2.89
♂ 5	14.6	12.2	15.2	15.2	15.2	18.0	10.4	13.4	4.41	2.53	1.88
6	20.2	14.2	18.6	22.2	14.3	20.2	9.0	11.8	15.38	14.10	1.28
7	14.0	12.2	13.6	13.8	15.6	15.6	11.4	13.0	3.72	1.96	1.76
8	15.2	10.0	17.0	20.8	20.0	17.0	13.0	14.0	2.67	3.76	−1.08

Block II

	♀ 1	2	3	4	5	6	7	8	W_r	V_r	$W_r - V_r$
1	24.2	16.2	30.8	27.0	20.2	16.8	14.4	16.0	14.02	25.39	−11.37
2	16.5	18.8	14.6	18.6	15.3	15.2	14.8	13.2	7.18	6.61	0.56
3	30.4	23.0	21.2	25.4	20.0	28.4	14.2	14.4	17.91	22.84	−4.93
4	17.8	13.0	16.3	18.0	14.2	14.8	12.2	11.2	11.10	10.19	0.91
♂ 5	18.8	13.6	15.4	13.8	15.2	16.0	12.2	20.0	5.73	4.82	0.91
6	23.4	14.0	14.8	17.0	17.3	22.6	10.2	12.8	16.57	18.35	−1.79
7	16.6	9.2	16.2	14.4	15.6	11.0	10.6	9.8	4.84	4.79	0.05
8	17.2	11.6	18.2	20.8	17.4	12.6	9.8	15.8	4.04	8.33	−4.29

in the progeny of the corresponding crosses. The tests of 4.1 confirm heterogeneity of $W_r - V_r$.

(i). The analysis of the variance of $W_r - V_r$ is

	Mean square	Df	P
Lines	31.67	7	.01–.001
Blocks	13.98	1	.05–.01
Error	2.46	7	

(ii). In the test of the deviation of the slope of the (V_r, W_r) line from unity, $t_{14} = 4.068$ and P = .01–.001, which is highly significant.

The significant variation of $W_r - V_r$ from line to line means that these diallel tables do not satisfy the hypotheses of section 2. The graph of $W_r - V_r$ against $_r$ reveals no trend so that a change of scale is not suggested. The source of the trouble is probably complementary gene action in the cross $\Theta_1 \times \Theta_3$, and an adjustment of the plot means of the progeny of this cross should make the diallel cross conform to the hypotheses.

The formulae of 4.9 provide estimates of the plot means in the diallel table of means over the two blocks. The estimate of the difference between any two corresponding plot means in the two blocks = (BI total − BII total)/$(n^2 - 2)$ which minimises the block interaction sum of squares in the analysis of variance of two diallel tables. (The totals, as in 4.9, exclude the means of the progeny of $\Theta_1 \times \Theta_3$). The estimated plot means are

Block	♀1 × ♂3	♀3 × ♂1
I	23.3	19.2
II	23.6	19.6

which are about two-thirds of the observed values. The other new entries in Table 3 are

Block	W_1	V_1	$W_1 - V_1$	W_3	V_3	$W_3 - V_3$
I	10.27	7.82	2.45	17.51	17.44	1.37
II	10.29	9.94	0.34	10.33	6.44	3.89

The tests now show $W_r - V_r$ to be uniform

(i). In the analysis of variance of $W_r - V_r$ the probabilities of both line and block mean squares are .20–.10.

(ii). The slope of the (V_r, W_r) line does not differ significantly from unity ($P > .90$).

6.3. We are now free to analyse the variance of the diallel tables and to

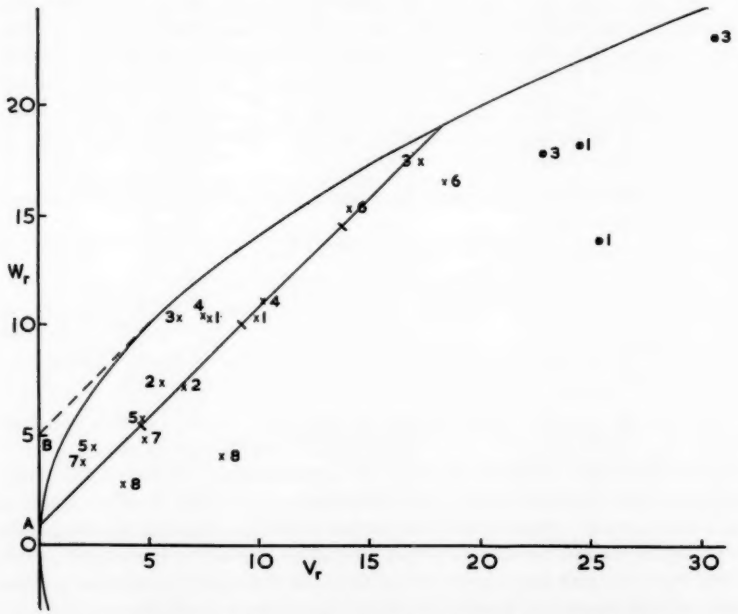

FIGURE 2.—(V_r, W_r) graph for flowering time in a replicated cross of eight inbred varieties of *Nicotiana rustica*. After adjusting the measurements of the progeny of the crosses of varieties 1 and 3, the points lie near a straight line of unit slope inside the limiting parabola $W_r^2 = 19.97V_r$. The unadjusted points (circled) lie well off this line. The three points of division and the ends of the line correspond to parents with 100%, 75%, 50%, 25% and 0% dominants (reading up from the bottom). The two blocks agree, except for line 3. AB/OB estimates H_1/D.

find the components of variation, remembering, of course, that our results provide no information about the cross $\Theta_1 \times \Theta_3$. Figure 2 depicts the points (V_r, W_r), the limiting parabola $W_r^2 = V_{0L0}V_r$, and the line of unit slope through the adjusted mean point (V_{1L1}, W_{0L01}). The unadjusted points lie well off this line.

The analysis of variance is derived from the adjusted table 3 in the way described by HAYMAN (l.c.). Table 4 contains the significance levels of the

TABLE 4

Significance levels of the components of variance derived from the data in table 3.

Item	Probability
(a) $D - F + H_1 - H_2$	$<.001$
(b_1) h^2	$<.001$
(b_2) $H_1 - H_2$	$>.20$
(b_3)	$<.001$
(b) H_2	$<.001$
(c) Reciprocal	$<.001$
(d) differences	$<.001$
(B) Blocks	$>.20$

components. The significance of (b) shows that dominance is present while (b_1) shows that it is largely unidirectional. From the sign of h we see that the progeny mean is less than the parental mean so that this dominance is in the direction of early flowering time. Asymmetry is not significant (b_2) at loci exhibiting dominance. This also implies that (a) detects purely additive variation and this is highly significant. The significant differences between reciprocal families are an unfortunate systematic effect due to faulty lay-out of seed-boxes in the greenhouse so that the use of mean reciprocals in all other computations is justified. The overall block difference is not significant. The block interaction mean square, which is the estimate of E, is 3.37.

6.4. The values of the statistics of 3.2 are

Block	V_{0L0}	W_{0L01}	V_{1L1}	V_{0L1}	$(m_{L1} - m_{L0})^2$
I	20.33	8.97	7.58	4.20	3.63
II	19.60	8.76	8.68	5.13	3.41

and the mean estimates of the components of variation with their standard errors are

$\hat{D}$	F	$\hat{H}_1$	$\hat{H}_2$	$\hat{h}^2$	$\hat{E}$	$D - \hat{H}_1$
16.59	−0.59	7.76	7.11	12.60	3.37	8.83
±1.79	±4.24	±4.12	±3.59	±2.40	±0.60	±3.54

The sum of the squares of deviations of observed from expected values of V_{0L}^0, V_{1L1}, V_r and W_r $(r = 1, \ldots n)$ is 142.84. The two diallel tables supply 37 statistics, and 12 constants are fitted to them, leaving 25 degrees of freedom for error. Hence the mean squared deviation is 5.71. Now, from the top left corner of the matrix in table 1, $\mathrm{Var}\hat{D} = \frac{1}{2}(n + 1)s^2/n$, the factor of $\frac{1}{2}$ allowing for the use in the estimation of means of statistics from two blocks. Since $n = 8$ and $s^2 = 5.71$, $\mathrm{Var}\hat{D} = 3.21$. Similarly, from the

next term down the leading diagonal of the matrix, $\text{Var}\hat{F} = \frac{1}{2} \times 6.28 \times 5.71 = 17.94$, etc. The square roots of these variances give the standard errors above. Their large values are due to the variations in V_3 and W_3 between blocks which suggest that this variety is unstable to environmental variations. Apparently $\hat{H}_1$ and $\hat{H}_2$ are not quite significant but the more reliable analysis of variance in 6.3 shows that $\hat{H}_2$ is significant. Here $\hat{D} - \hat{H}_1$ is just significantly different from zero; it is highly so if we use the simple formula for $\text{Var}(\hat{D} - \hat{H}_1)$ at the end of 3.2 which gives a standard error of 1.96 for $\hat{D} - \hat{H}_1$. Evidently dominance is present but it is definitely not complete dominance.

6.5. $(\hat{H}_1/\hat{D})^{\frac{1}{2}} = 0.68$ is an estimate of the mean degree of dominance over all loci. In figure 2, AB/OB estimates H_1/D without allowing for E. If the formulae of 3.2 are applied to the original diallel tables (i.e. without adjusting the cross $\Theta_1 \times \Theta_3$) then $(\hat{H}_1/\hat{D})^{\frac{1}{2}} = 1.02$. This illustrates the extent to which nonallelic genic interaction can inflate this estimate of the degree of dominance and emphasises the importance of the preliminary survey with the (V_r, W_r) graph.

The estimate of uv is $\hat{H}_2/4\hat{H}_1 = 0.23$ agreeing with the result in the analysis of variance (6.3) that H_1 is not significantly different from H_2.

$\{(4\hat{D}\hat{H}_1)^{\frac{1}{2}} + \hat{F}\}/\{(4\hat{D}\hat{H}_1)^{\frac{1}{2}} - \hat{F}\} = 0.95$ which is near enough to unity, implying equality between the numbers of dominant and recessive alleles in the parents. This is, of course, a necessary consequence of the foregoing result that $u_i = v_i = \frac{1}{2}$.

$\hat{h}^2/\hat{H}_2 = 1.77$ so that at least two of the genes controlling flowering time exhibit some degree of dominance.

6.6. The order of dominance of the parents, determined by $W_r + V_r$, is 75821436 and the order of flowering time is 78524631, parent 7 being the earliest and carrying the most dominants. The correlation between y_r and $W_r + V_r$ is 0.80 corroborating our result above (6.3) that early flowering is partially dominant.

6.7. This example has been worked in detail more to illustrate our methods and some of the difficulties which arise than to present a set of results conforming closely to our theories. Measurements of height and leaf length from the same plants were more consistent than flowering time over the two blocks and showed less or no reciprocal differences and little genotype-environment interaction. Further, while height exhibited genic interaction, leaf length satisfied our hypotheses completely.

SUMMARY

Experiments with diallel crosses provide a powerful method of investigating polygenic systems. The theory of a diallel cross between homozygous lines is discussed in terms of components of variation similar to MATHER'S (l.c.) D and H components. Assuming a simple underlying genetical system, we show that the various statistics obtained from measurements on the progeny provide estimates of the overall degree of dominance, of the rela-

tive dominance properties of the parents, and of the symmetry or otherwise of the gene distribution in the lines. The dominance relations are exhibited graphically. The effects of complications such as genic interaction are also considered. A genetic algebra is used to simplify the mathematical computations. The final sections are a summary of the method of analysis and a worked example.

LITERATURE CITED

HAYMAN, B. I., 1954 The analysis of variance of diallel crosses. Biometrics **10:** 235–244.

HAYMAN, B. I. and K. MATHER, 1955 The description of genic interaction in continuous variation. Biometrics (in press).

HULL, F. H., 1945 Regression analyses of yields of hybrid corn and inbred parent lines. Maize Genetics News Letter **19:** 21–27.

1952 Overdominance and recurrent selection. Heterosis, chap. 28, Iowa State College Press, Ames, Iowa.

JINKS, J. L., 1954 The analysis of heritable variation in a diallel cross of *Nicotiana rustica* varieties. Genetics **39:** 767–788.

JINKS, J. L. and B. I. HAYMAN, 1953 The analysis of diallel crosses. Maize Genetics News Letter **27:** 48–54.

MATHER, K., 1949 Biometrical Genetics. London, Methuen and Co.

MATHER, K. and A. VINES, 1952 The inheritance of height and flowering time in a cross of *Nicotiana rustica*. Quantitative Inheritance, 49–80, H.M.S.O., London.

WRIGHT, S., 1952 The genetics of quantitative variability. Quantitative Inheritance, 5–41, H.M.S.O., London.

MODIFICATION AND DOMINANCE OF THE GENE GOVERNING FORMATION OF HIGH CONCENTRATIONS OF *BETA*-CAROTENE IN THE TOMATO[1]

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FROM the progeny of crosses between normal canning tomatoes, *Lycopersicon esculentum* Mill., and the wild species *L. hirsutum* Humb. and Bonpl., LINCOLN *et al.* (1943) and KOHLER *et al.* (1947) isolated a new tomato type which was distinguished from normal tomatoes by producing orange fruits in which most of the carotene produced was *beta*-carotene. From the study of a cross between the red canning variety Indiana Baltimore and a true breeding, high *beta*-carotene orange strain, LINCOLN and PORTER (1950) postulated that in high *beta*-carotene orange tomatoes the shift from red (lycopene) to orange (*beta*-carotene) depended upon a single gene *B* which lacked dominance. TOMES *et al.* (1953) demonstrated that the gene *B* was not an allele of either *R/r* (red/yellow flesh, high/low total carotene) or *T/t* (red/tangerine flesh, lycopene/prolycopene, *zeta*-carotene type) and that in the F_1 and F_2 generations of crosses involving the *beta* orange type with yellow-fleshed or tangerine-fleshed types the gene *B* appeared to behave as originally postulated. These authors noted, however, that considerable evidence was available which inferred that *B* might actually be a dominant gene and that the F_1 and F_2 values reported might be accounted for by modification. The purpose of the present paper is to clarify the dominance of the alleles *B/b* and the mode of inheritance of high concentrations of *beta*-carotene in the tomato.

INCONSISTENCIES IN PRESENT GENETIC VIEWPOINT

Normal red tomatoes produce major quantities of lycopene, a small quantity of *beta*-carotene, and traces of certain other carotene pigments, particularly *gamma*-carotene. The same pigments are found in high *beta* orange tomatoes, but *beta*-carotene represents the major fraction. In the cross reported by LINCOLN and PORTER (1950) the high *beta* parent averaged 93% of total carotene as *beta*-carotene, the red parent averaged 10%, and the F_1 generation averaged 61%. The F_2 population of 209 plants gave a complete array of *beta*-carotene values, but three distinct peaks were observed in the distribution frequencies. These peaks occurred at 12, 55 and 88%. Low frequencies were found between 20 and 30% and between 70 and 80%. Class limits of 0–24.9%, 25–74.9% and 75–100% *beta*-carotene were assigned to the red,

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the intermediate, and the *beta* orange phenotypes, and it was demonstrated that the frequencies in each group might logically have been derived from a population consisting of *bb, Bb* and *BB* in the ratio of 1:2:1. According to theory then, plants producing fruits containing high concentrations of *beta*-carotene, for example 90 to 95%, should breed true for this character, while plants producing intermediate quantities of lycopene and *beta*-carotene should segregate in the above manner.

That this interpretation of the data might be inadequate was noted in the course of a breeding program undertaken to incorporate this high provitamin A content into tomatoes of commercial type. First, it was noted that certain selections which were intermediate in phenotype bred true for the intermediate conditon. Second, while certain selections which were high in *beta*-carotene content did breed true, other selections segregated a few red-fruited plants in row progeny tests.

METHODS

The analytical methods used for the determination of pigment quantities in the present study have been reported elsewhere (TOMES *et al.* 1953) along with quantitative data and diagrammatic chromatograms of the parent pigment types. All of the observations to be reported in the present paper involve advanced generation material derived by selection from crosses between high *beta*-carotene orange selections and commercial type red tomato varieties.

RESULTS AND DISCUSSION

A careful evaluation of all of the progenies available in the 1952 breeding nursery demonstrated that six types of progenies resulted from selections having intermediate or high *beta*-carotene values:

1. All high *beta*-orange.
2. All intermediate, orange-red.
3. Red, orange-red, and orange in approximately a 1:2:1 ratio.
4. Approximately 3 orange-red to 1 red.
5. Approximately 3 orange to 1 red.
6. Approximately 3 orange-red to 1 orange.

Small progenies of from 19 to 30 plants of each of the six types were analyzed chemically in order to check visual classifications. For example, progeny 1002 was derived from a parent selection which contained 94% beta-carotene. Of 30 plants analyzed, all contained over 85% *beta*-carotene and no visual color segregation was noted—type 1, above. Progeny 1134 was derived from a selection which contained 51% *beta*-carotene. Twenty plants all gave intermediate values ranging from 46 to 71%—type 2. Progeny 1104 was derived from a selection containing 52% *beta*-carotene and included 3 red-fruited plants, 11 intermediates, and 6 high *beta* orange plants by analysis—type 3. An intermediate selection with 34% *beta*-carotene produced progeny 1064 which included 14 intermediate plants and 6 plants which produced red fruit —type 4. A selection assayed at 91% *beta*-carotene produced progeny 1027

containing 17 plants with values above 86% and 13 plants with values below 20%—type 5. Progeny 1079 was derived from a selection which contained 78% *beta*-carotene (slightly above the class limit set for an intermediate type) and produced 6 plants with values above 78% and 13 with values in the intermediate class ranging from 43 to 73%—type 6. It should be noted that, although most of the progenies available were rather small, many of the progenies which were classified visually approached the approximate ratios cited more closely than did certain of those analyzed. *Beta*-carotene values were available from prior determinations for each of the parent selections. Definitely high *beta* selections, i.e., those containing over 85% *beta*-carotene, gave rise only to progenies of type 1 or type 5. The other progenies were all derived from intermediate parents or, as is the case with progeny 1079 cited above, from progenies where the parental values were close to the class limit set by LINCOLN and PORTER on the basis of their F_2 distribution.

All of these apparently aberrant results may be explained by the following assumptions:

1. The allele *B* is dominant. *BB* or *Bb* act to produce *beta*-carotene in quantity. The *bb* genotype produces mainly lycopene.

2. Commercial red strains possess at least one major modifier or inhibitor of *B* (I^B for the purpose of discussion) which partially inhibits the action of *B* in producing *beta*-carotene at the expense of lycopene. When I^B is present in either homozygous or heterozygous form, the action of *B* is partially inhibited, yielding a plant which produces intermediate quantities of *beta*-carotene.

3. *B* and I^B are inherited independently.

On this basis, the 93% *beta*-carotene, true breeding parent used by LINCOLN and PORTER was of the genotype BBi^Bi^B; the red parent bbI^BI^B, and the F_1 BbI^Bi^B. If the expression of *B* is dependent upon the alternative states of I^B/i^B, the F_2 ratio should be 4 red (bbI^B- or bbi^Bi^B) : 9 intermediate (B-I^B-) : 3 orange (B-i^Bi^B). This 4 : 9 : 3 ratio is so close to the 1 : 2 : 1 ratio postulated that differentiation on a statistical basis is extremely difficult. The chi square values are such that LINCOLN and PORTER's F_2 might have been drawn from either population. In addition, LINCOLN and PORTER's F_2 distribution shows considerable overlap at the class limit between the intermediate and the high *beta* type. That such overlap may well exist is demonstrated by progeny 1079 cited above and by progenies of other similarly classed parents. This means that some misclassification probably occurred with regard to orange-red and orange types. Moreover, the F_2 ratios involving *r* and *B*, and *t* and *B*, reported by TOMES *et al.* (1953) would fit the modification scheme presented if only a few such questionable plants were misclassified. In these crosses, the gene *B* appeared to act as originally postulated in the presence of *R* and *T*.

From the above it is apparent that high *beta* selections may either breed true, or segregate orange and red-fruited plants depending upon whether *B* is homozygous or heterozygous (progeny types 1 and 5). In like manner, intermediate parents may give rise to any of the other four types of progenies.

Additional support for this interpretation as regards *B* was derived from an analysis of the F_1 plants of crosses involving parents with over 90% *beta*-carotene and commercial type red strains. These F_1 progenies, although small, were of two types—those in which all plants were intermediate orange-red, and those which segregated orange-red and red in approximately a 1 : 1 ratio. In a few instances where segregation of the F_1 generation was observed, a selfed progeny of the original high *beta* parent was available in the 1952 field. These selfed progenies segregated for orange and red-fruited plants, again demonstrating that *B* may be either homozygous or heterozygous in a plant producing above 90% *beta*-carotene. No obvious visual or chemical differentiation between *BB* and *Bb* high *beta* plants was detected.

Although numerous crosses between red types and true breeding high *beta* types had been made, and at least one involving high *beta* and tangerine (*tt*), and one involving high *beta* and yellow (*rr*), all F_1 plants produced prior to 1952 were of the intermediate phenotype. Proof of the analysis with regard to *B* appeared to be the demonstration that a high *beta* F_1 plant could be produced by crossing a high *beta* orange with a red strain. If the above analysis is correct, certain red-fruited plants should lack the modifier. For example, all red-fruited plants from the progeny of a high *beta* selection which segregates only red and orange should be of the genotype bbi^Bi^B. Several of these red segregates were crossed with a true breeding high *beta* strain and the F_1 plants, as well as the selfed progenies of the red parents, were analyzed in the 1953 field. The results of these crosses and of certain other crosses of interest are summarized in table 1. The percentages reported represent percent of total carotene as determined first by the direct reading method on crude pigment extracts and second by the addition of the *beta*-carotene, *gamma*-carotene, and lycopene fractions after separation by chromatography. The earlier work recorded only the values obtained by the direct reading method.

The first three items in table 1 represent samples from progenies derived from red-fruited segregates lacking the modifier. These progenies bred true for red color and were reasonably consistent chemically. The F_1 progenies reported in the next four items of the table represent crosses involving these bbi^Bi^B red-fruited plants with true breeding high *beta* strains. By either analytical method the values obtained were all above 83%. Such values are comparable to those consistently obtained for true-breeding high *beta* strains, thus demonstrating the dominance of *B* in the absence of the modifier. Cross 5377 and its reciprocal 5378 represent crosses between a true breeding high *beta* strain and a true breeding intermediate. The introduction of the modifier from the intermediate parent results in an obvious drop in the proportion of *beta*-carotene.

In cross 5379 a true breeding high *beta* was crossed with the red variety Rutgers, while in cross 5393 a consistent intermediate was crossed with a red selection lacking the modifier. Both 5379 and 5393 should yield F_1 plants heterozygous at both loci. Both produced fairly comparable values. Cross 5381 involved a consistent intermediate with the variety Rutgers.

TABLE 1

The relative proportions of beta-carotene and of beta-carotene, gamma-carotene and lycopene in certain test hybrids and selections of tomatoes as determined by two methods.

Cross or Selection	Genotype	No. of plants analyzed	Direct readings		Chromatography					
			% beta-carotene		% beta-carotene		% gamma-carotene		% lycopene	
			Mean	Range	Mean	Range	Mean	Range	Mean	Range
50143F5-7-22-1 (bbi^Bi^B red selection)	bbi^Bi^B	8	16.5	11.6–26.8	13.7	5.4–25.0	2.0	0.4–3.8	84.3	71.2–93.7
50143F5-7-32-1 (bbi^Bi^B red selection)	bbi^Bi^B	10	11.4	3.1–17.7	11.8	6.3–19.1	1.7	0.0–3.1	86.6	79.4–92.5
50143F5-7-38-2 (bbi^Bi^B red selection)	bbi^Bi^B	7	16.1	11.6–23.0	16.6	9.3–24.4	1.7	0.9–2.6	81.7	73.4–89.4
5374F1 ($bbi^Bi^B \times BBi^Bi^B$) (red × orange)	Bbi^Bi^B	10	88.2	85.6–89.5	94.0	90.3–98.1	4.3	0.0–6.7	1.7	0.0–4.5
5375F1 ($bbi^Bi^B \times BBi^Bi^B$) (red × orange)	Bbi^Bi^B	10	89.8	87.2–92.5	94.2	92.2–99.3	4.5	0.0–7.0	1.3	0.2–2.5
5376F1 ($bbi^Bi^B \times BBi^Bi^B$) (red × orange)	Bbi^Bi^B	10	89.8	83.4–96.3	93.7	90.8–96.4	4.6	2.5–5.4	1.7	0.4–3.8
5380F1 ($BBi^Bi^B \times bbi^Bi^B$) (orange × red)	Bbi^Bi^B	4	87.0	86.4–87.2	91.3	83.1–95.7	4.3	3.6–4.9	4.3	0.6–12.3
5377F1 ($BBi^Bi^B \times BBl^Bl^B$) (orange × orange-red)	BBl^Bi^B	10	74.0	53.6–82.6	80.6	59.2–92.8	5.7	3.6–9.2	13.8	1.6–31.6

TABLE 1 (*continued*)

Cross or Selection	Genotype	No. of plants analyzed	Direct readings		Chromatography					
			% beta-carotene		% beta-carotene		% gamma-carotene		% lycopene	
			Mean	Range	Mean	Range	Mean	Range	Mean	Range
5378F1 ($BBl^{B}l^{B} \times BBi^{B}i^{B}$) (orange-red × orange)	$BBl^{B}i^{B}$	10	78.2	46.7–91.0	79.4	73.5–84.6	6.2	2.6–12.2	15.4	7.5–23.6
5379F1 ($BBi^{B}i^{B} \times bbl^{B}l^{B}$) (orange × red)	$Bbl^{B}i^{B}$	10	62.6	43.6–81.8	61.6	46.3–75.8	6.6	5.3–8.1	31.8	18.9–47.1
5393F1 ($BBl^{B}l^{B} \times bbi^{B}i^{B}$) (orange-red × red)	$Bbl^{B}i^{B}$	9	55.9	45.9–67.1	61.5	50.0–77.6	5.1	0.0–8.4	33.4	16.9–44.1
5381F1 ($BBl^{B}l^{B} \times bbl^{B}l^{B}$) (orange-red × red)	$Bbl^{B}l^{B}$	10	45.7	30.7–55.9	51.3	33.6–64.4	6.0	2.5–8.4	42.7	28.7–62.4
Rutgers (red variety)	$bbl^{B}l^{B}$	10	8.8	6.2–11.6	8.1	3.1–12.9	1.8	1.0–3.1	90.1	85.0–95.7

A consideration of the data presented in table 1 with regard to the various intermediate F_1 types leads to certain difficulties. For example, crosses 5377 and 5378 yield somewhat higher values than do other intermediate F_1 progenies. In the absence of the modifier, *B* has been demonstrated to be completely dominant. These data suggest that *BB* and *Bb* may produce slightly different results in the presence of the modifier. In like manner, $5381F_1$ gave somewhat lower values than $5379F_1$ and $5393F_1$, suggesting that I^BI^B and I^Bi^B may produce slightly different results in the presence of *B*. These interactions are subject to question, however, for the possibility of additional minor modifiers has not been eliminated.

Table 1 also reports the analysis of 10 plants of the variety Rutgers. It is interesting to note that the progenies of the three red selections lacking the modifier averaged slightly higher than Rutgers in *beta*-carotene content. This suggests that the modifier gene exhibits some influence on the production of *beta*-carotene in normal plants homozygous for *b*.

One other point is of interest in connection with the data reported in table 1. Where appreciable quantities of *beta*-carotene are present, the *beta* values reported by chromatography are usually slightly higher than those reported by the direct reading method. The method utilizing readings of the crude pigment extract assumes that *beta*-carotene and lycopene are present to the near exclusion of other pigments. When the pigments are separated and calculated by chromatographic methods, it appears that the intermediate and the high *beta* F_1 plants contained more *gamma*-carotene than did high lycopene strains. Since *gamma*-carotene is a structural intermediate between lycopene and *beta*-carotene, this is suggestive of a conversion of lycopene to *beta*-carotene by ring closure mediated by the gene *B*. Moreover, the dominance of *B* in the absence of the modifier tends to support PORTER and LINCOLN's (1950) contention that the direction of synthesis is from lycopene to *beta*-carotene. This evidence does not rule out the possibility of parallel synthesis as has been suggested by GOODWIN (1952), GOODWIN and JAMIKORN (1952), and JENKINS and MACKINNEY (1953). Evidence to date from incubation experiments on high *beta* tomatoes is contradictory. MANUNTA (1953) reports that incubation at 36°C inhibits lycopene formation, but fails to inhibit *beta*-carotene formation in high *beta* strains. WALLACE (1953), on the other hand, reports that incubation at 33°C inhibits lycopene formation in the variety Rutgers, and *beta*-carotene formation in high *beta* strains of the type discussed. If MANUNTA's report is correct, the role of lycopene as a possible precursor for *beta*-carotene in the tomato is difficult to rationalize even in specially bred high *beta* strains.

From a practical standpoint the present work suggests that varying the genotype with respect to *B* and its modifier provides four types of true breeding tomatoes, each possessing a different level of provitamin A content.

SUMMARY

In the tomato the alleles *B*/*b* govern the occurrence of high or low concentrations of *beta*-carotene. The gene was originally designated as an incom-

plete dominant. Evidence is presented to show that *B* is dominant, and that the intermediate nature of the F_1 progeny, as well as the F_2 distribution, may be explained by the interaction of *B* with a modifier contributed by the low *beta* (red) parent. The F_2 ratio on this basis should be 4 red (high lycopene) : 9 intermediate : 3 orange (high *beta*-carotene). The expected advanced generation progenies have been derived. The red genotype lacking the modifier, when crossed with true-breeding high *beta* strains, produces an F_1 type containing concentrations comparable to the high *beta* parent, demonstrating the dominance of *B* in the absence of the modifier. Preliminary data indicate that red strains lacking the modifier produce slightly more *beta*-carotene than do types such as Rutgers. If true, four levels of provitamin A content are available in the various genotypes homozygous for the two factors. The significance of the data reported with regard to the biosynthesis of carotenes in the tomato is considered briefly.

LITERATURE CITED

GOODWIN, T. W., 1952 Studies in carotenogenesis. 3. Identification of the minor polyene components of the fungus *Phycomyces blakesleeanus* and study of their synthesis under various cultural conditions. Biochem. J. **50:** 550–558.

GOODWIN, T. W., and M. JAMIKORN, 1952 Biosynthesis of carotenes in ripening tomatoes. Nature **170:** 104–105.

JENKINS, J. A., and G. MACKINNEY, 1953 Inheritance of carotenoid differences in the tomato hybrid yellow × tangerine. Genetics **38:** 107–116.

KOHLER, G. W., R. E. LINCOLN, J. W. PORTER, F. P. ZSCHEILE, R. M. CALDWELL, R. H. HARPER and W. SILVER, 1947 Selection and breeding for high *beta*-carotene content (provitamin A) in the tomato. Bot. Gaz. **109:** 219–225.

LINCOLN, R. E., F. P. ZSCHEILE, J. W. PORTER, G. W. KOHLER and R. M. CALDWELL, 1943 Provitamin A and vitamin C in the genus Lycopersicon. Bot. Gaz. **105:** 113–115.

LINCOLN, R. E., and J. W. PORTER, 1950 Inheritance of *beta*-carotene in tomatoes. Genetics **35:** 206–211.

MANUNTA, C., 1953 Analisi genetica della popolazione F_3 degli incroci fra *L. esculentum* e *L. hirsutum*. Selezione di varieta di pomodori caratterizzati da alto contenuto in provitamina A e vitamina C. Genet. Agr. 1953: **3:** 235–263.

PORTER, J. W., and R. E. LINCOLN, 1950 I. Lycopersicon selections containing a high content of carotenes and colorless polyenes. II. The mechanism of carotene biosynthesis. Arch. Biochem. **27:** 390–403.

TOMES, M. L., F. W. QUACKENBUSH, O. E. NELSON, JR. and BETTY NORTH, 1953 The inheritance of carotenoid pigment systems in the tomato. Genetics **38:** 117–127.

WALLACE, V., 1953 The chemistry of phytofluene and its relationship to other carotenes of the tomato. Ph.D. Thesis, Purdue University, Lafayette, Ind.

IMMUNOGENETIC STUDIES OF TYROSINASE SPECIFICITY

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MEASUREMENTS of tyrosinase activity in mycelial extracts of the fungus Glomerella have shown wide variations in activity which are correlated with genotype, with variations in the environmental conditions under which the organism is grown (MARKERT 1950), or with the age of the mycelium (SUSSMAN and MARKERT 1953). Any one or a combination of two or more of the following hypotheses might be advanced to explain these differences in tyrosinase activity: 1) The various mycelia may synthesize different quantities of tyrosinase. 2) The enzymatic efficiency of the tyrosinase molecule might be altered, particularly by the genetic changes. 3) Enzymatic activity might be altered by changes in associated substances (e.g., inhibitors) regulating the effective activity of the tyrosinase molecule.

Serological examination of protein extracts of Glomerella was undertaken in an effort to test these hypotheses and in particular to examine the possibility that genetically induced changes in tyrosinase activity might have altered the structure of tyrosinase in a serologically detectable fashion. Serological properties of tyrosinase from standard type Glomerella extracts have previously been described (OWEN and MARKERT 1954). In these previous studies Glomerella tyrosinase proved to be an effective antigen in rabbits. Antibodies against tyrosinase rapidly inactivated the enzyme and eventually precipitated the enzyme from solution. The enzymatically active surface of the tyrosinase molecule is strongly implicated in these antibody-tyrosinase reactions, because of the rapid inactivation of the enzyme in the presence of antibody and because of the effects on inactivation produced by varying the relative concentrations of enzyme, substrate, and antibody. The present report gives results of an extension of these serological tests to extracts derived from mycelia of different genotypes grown under various environmental conditions. An examination was also made of the response of tyrosinase from other species (Neurospora, Solanum, Psalliota and Tenebrio) to antisera against Glomerella tyrosinase.

MATERIALS AND METHODS

Glomerella cultures. The standard type Glomerella culture is designated A^1B^1 and differs as indicated by changes at two loci from wild type Glomerella (A^+B^+) (see WHEELER and MCGAHEN 1952 for terminology). Mutations were induced in standard type conidia by ultraviolet irradiation, X-irradiation,

[1] This work was largely performed while the first author was a Merck Fellow of the National Research Council.

or neutron bombardment (MARKERT 1952). Mutants exhibiting changes in melanin formation were then screened for possible variations in the tyrosinase content of their mycelia. Several mutants with tyrosinase activities varying from almost no activity to greatly enhanced activity were then selected for the preparation of extracts to be examined serologically.

Preparation of extracts. The procedure generally used in preparing extracts was as follows: Small mycelial transplants from each Glomerella type were inoculated to the center of about 50 Petri dishes (100 × 15 mm) each containing 30 ml of complete medium (see MARKERT 1950 for media). The cultures were then grown at 25°C for 10 days by which time the mycelia had generally spread over the entire surface of the medium. The harvested mycelia were freed from the agar medium and ground in a phosphate buffer (pH 7.0) in a colloid mill for 20 to 30 min. The resulting brei was centrifuged at 13,000 g for 30 min. and the supernatant then dialyzed against buffer for 24 hrs. All extractive procedures were carried out in the cold. The dialyzed supernatant was frozen and lyophilized, and the resulting dry powder when redissolved was used as the extract for all enzyme and serological tests.

In addition to the surface cultures, submerged aerated liquid cultures of several of the Glomerella types were also prepared. Extracts were made from these liquid cultures according to the procedures employed for the surface cultures.

Neurospora (mutant no. 84605a, cf. HOROWITZ and SHEN 1952) extracts were prepared in the same way except that the mycelia were obtained exclusively from liquid cultures. In these liquid cultures the medium was distributed in 20-ml aliquots to 125-ml flasks and was made up of minimal medium supplemented with 10 γ tyrosine and 2 mg cysteine per 20 ml of medium. Cultures were grown for 10 days at 25°C with no shaking or aeration. The Neurospora mycelia floated on the medium in contrast to the Glomerella which grew submerged in liquid culture.

Extracts of Solanum, Psalliota and Tenebrio were made by grinding frozen tissue with a mortar and pestle, washing with acetone, and then suspending the acetone-dried powders in buffer. The suspensions were then centrifuged at 13,000 g for 30 min. and the remainder of the extractive procedure carried out as outlined for the Glomerella cultures.

Enzyme measurement. Tyrosinase activity was measured in a Klett-Summerson colorimeter equipped with a No. 42 (blue) filter; the rate of pigment (dopachrome) formation proved to be a satisfactory index of the concentration of tyrosinase. The reaction mixture generally contained 0.5 ml of .02 M L dopa, 1 ml of serum, 1 ml of enzyme solution, and 3 ml of buffer (pH 7.0) to make a total volume of 5.5 ml. Readings were taken at 10-second intervals beginning 10 seconds after mixing and the tyrosinase activity was calculated on the basis of the maximum reaction achieved in 40 seconds.

Throughout this report tyrosinase activity measurements are recorded in terms relative to the activity of the standard type extract (S) which is given an index value of 100. In absolute terms, one milligram of extract (S) reacting

in a volume of 5.5 ml containing an excess of dopa produced a change of one unit on the Klett colorimeter scale in 5.7 seconds. Accordingly, one milligram of extract S-3 with an index value of 1000, would produce a change of 10 colorimeter units in 5.7 seconds or of 1 unit in 0.57 seconds.

Preparation of antisera. The extracts, at a concentration of 10 mg of the dried powder per ml of physiological saline or distilled water, were injected intravenously into rabbits, 0.5 ml per injection. After 9 injections (Monday, Wednesday and Friday of three successive weeks) the rabbits were given a 10-day rest, then bled by cardiac puncture. Usually only one bleeding, of 50 ml whole blood, was taken at this time. After a further rest of at least two weeks the rabbits were reimmunized with an intraperitoneal injection of 2 ml of the extract at 10 mg/ml, then two intravenous injections of 0.5 ml on alternate days thereafter. Bleedings on the tenth and eleventh days after the last injection provided the " Number 2," or reimmunization antisera. Later reimmunizations to provide " Number 3 " and " Number 4 " antisera were similarly performed.

RESULTS

The details of preparative procedures are important if quantitative comparisons are to be made of the enzyme activities of different preparations. It has previously been noted that the tyrosinase activity in a Glomerella mycelium is dependent upon such environmental conditions as the nutrient composition of the medium, temperature, and the nature of growth—whether surface or submerged (MARKERT 1950). Temperature and nutrition also affect tyrosinase

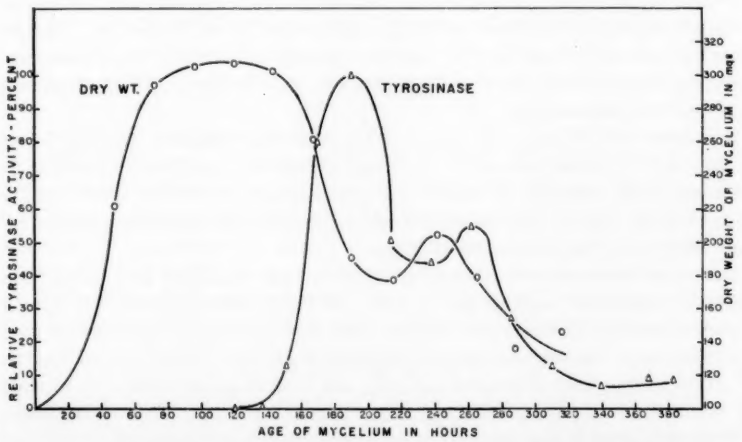

FIGURE 1.—Curves showing the formation of tyrosinase during growth of the mycelium. Growth measured as dry weight of the mycelium on a single Petri dish (15 × 100 mm) containing 30 ml of complete medium. Growth initiated by flooding surface medium with a dense spore suspension. Extracted tyrosinase activity measured colorimetrically. Points on tyrosinase curve are averages of three experiments.

activity in Neurospora (HOROWITZ and SHEN 1952). The age or stage of growth of the mycelium is of crucial importance in Glomerella. SUSSMAN and MARKERT (1953) found that tyrosinase synthesis starts at the time growth ceases, increases rapidly to a maximum and then declines to a much lower, relatively stable, level (fig. 1). The growth conditions for Glomerella cultures used in this investigation differ somewhat from those employed by SUSSMAN and MARKERT; nevertheless the mycelia were generally harvested near the peak of their tyrosinase activity in accord with the cultural conditions under which they were grown. However, growth conditions and the time of harvesting were varied for several standard type cultures with the result that extracts of different enzyme activities were obtained. These cultures of identical genotype (A^1B^1) are designated in table 1 as A^1B^1(S), A^1B^1(S-1), A^1B^1(S-2), A^1B^1(S-3). The A^1B^1(S) culture was grown under the same conditions as the mutant types on solid medium and is therefore used as a standard for comparing tyrosinase activities.

The type of growth, whether surface or submerged, appears to exert the most striking effect on the tyrosinase activity of the mycelium. Submerged cultures fail to develop large amounts of activity, although mutants with very high activity in surface culture (e.g., A^1B^1 318) do develop a small amount of tyrosinase activity in submerged culture (table 1).

Recently HOROWITZ and FLING (1953) in an investigation of Neurospora tyrosinase found that the effects of temperature on tyrosinase activity depended

TABLE 1

Relative tyrosinase activity of Glomerella mutants.

Mutant designation	Growth medium*	Tyrosinase activity**	Antisera	Antibodies induced
$A^1B^1$308	solid	6570	DH	+
$A^1B^1$318	solid	3100	DL	+
$A^1B^1$318	liquid	10	DI	0
$A^1B^1$65	solid	1100	DD	+
A^1B^1(S-3)	solid	1000	....	−
$A^1B^1$537	solid	985	DF	+
A^1B^1(S-2)	solid	970	BT	+
A^1B^1(S-1)	solid	642	CR	+
A^1B^1(S) standard	solid	100	DJ	0
A^1B^1	liquid	< 1	CT	0
$A^1B^1$218	solid	48	DG	0
$A^1B^1$212	solid	8	DK	0
$A^1B^1$216	solid	5	DM	0
$A^1B^1$216	liquid	< 1	CQ	0
$A^1B^1$503	solid	4	DE	0
84605a (Neurospora)	liquid	3	DN	0
$A^1B^1$507	solid	< 1	DA	0
$A^1B^1$507	liquid	< 1	CP	0

*Solid medium produced surface growth and liquid medium submerged growth except for Neurospora which grew partly submerged and partly on the surface.

**Relative activity calculated with the standard type (A^1B^1) as 100.

upon the strain used, some strains producing tyrosinase when grown at 35°C and others not. All the Glomerella mutants so far tested respond alike to temperature, that is, high temperatures drastically inhibit the development of tyrosinase activity under conditions otherwise conducive to maximum activity. Consequently all the cultures used in this investigation were grown at 25°C, a temperature conducive to tyrosinase development.

The effects of nutrition on tryrosinase development are complex and not consistent for all genotypes. That is, a nutritionally minimal medium supports the formation of high tyrosinase activity in mycelia of some genotypes while causing low activity in others. For most genotypes a complete medium was most conducive to the development of the maximum tyrosinase activity and this type of medium was therefore used in the agar Petri-dish cultures. A minimal medium was used for the liquid cultures in which a low tyrosinase activity was desired.

TABLE 2

*Tyrosinase activity of extract S-3 in presence of extracts from mutants with low activity.**

	Extract designation					
	A^1B^1503	A^1B^1218	A^1B^1318**	A^1B^1212	A^1B^1216	A^1B^1**
Δcolorimeter reading at 2 min.	89	138	104	78	81	88
Activity of added extract	5	46	17	5	9	1
Activity due to extract S-3	84	92	87	73	72	87

*Reaction medium composed of 1 ml (at 0.8 mg/ml) of extract S-3 from standard type, 1 ml (at 20 mg/ml) of low activity extract, 1 ml M/50 DL dopa, 2 ml buffer. Pigment formation measured in a Klett colorimeter as discussed in *Methods*.

**Cultures grown in liquid medium.

In comparing the enzymatic activity of the extracts from the various genetic types shown in table 1, the standard type has been assigned arbitrarily an activity of 100. It thus becomes apparent that under identical environmental conditions, single gene changes may reduce tyrosinase activity to essentially zero (mutant A^1B^1 507) or increase the activity over 65 times (mutant A^1B^1 308).

The wide variations in tyrosinase activity in these extracts is apparently due to large molecules since dialysis of the extracts does not change their relative activity. Variations in tyrosinase activity in extracts must be attributed either to variations in the number or kinds of tyrosinase molecules or to the presence of other large molecules which act as inhibitors. The possibility that low levels of tyrosinase activity in some extracts are due to the presence of inhibitors may be tested by mixing an active extract with the relatively inactive extracts. Accordingly, 1 ml of extract S-3 from standard type (at 0.8 mg/ml) was mixed with 1 ml (at 20 mg/ml) of a variety of relatively inactive extracts.

After one hour at 37°C the mixtures were placed overnight at about 4°C, then centrifuged and the supernatants added to 1 ml M/50 DL dopa + 2 ml of buffer. The pigment formation at 2 minutes for each mixture is given in table 2. These readings are corrected by subtraction of the small activity present in the extracts with low activity. Although some variation in effective activity of the S-3 extracts is thus apparent in the presence of a variety of extracts with low activity, this variation shows no correlation with the level of activity found in the low activity extract. It may reasonably be concluded, therefore, that large molecular inhibitors are not a significant factor in reducing the activity formed in these mutant extracts. This conclusion is further supported by serological data presented later in this report.

In a previous paper OWEN and MARKERT (1954) demonstrated that antiserum made against standard type Glomerella extracts will inactivate and precipitate the tyrosinase in such extracts. Accordingly, antisera were produced against extracts from various mutants exhibiting a wide range of tyrosinase activities and also from the standard type grown on surface and in submerged

TABLE 3

Antibody activity of antisera.

Antiserum	Inducing extract	Tyrosinase activity of extract	Antibody activity* of antiserum
CR_4	A^1B^1(S-1)	642	1000
DD_2	$A^1B^1$65	1100	1300
DF_2	$A^1B^1$537	985	2400
DH_2	$A^1B^1$308	6570	6000

*Measured in terms of the number of tyrosinase units inactivated by 1 ml of the antiserum under standard conditions.

cultures. These antisera were then tested for enzyme inactivating and precipitating ability. All of the antisera produced an extensive precipitation when mixed with any of the extracts. However, the presence of specific antibodies against tyrosinase in an antiserum appears to depend upon the level of tyrosinase activity in the inducing extract. It may be seen in table 1 that all extracts with tyrosinase activities of less than 642 failed to induce antibodies to tyrosinase even though some tyrosinase was contained in the injected extract. Furthermore, the antibody activity of an antiserum appears to depend somewhat upon the level of enzymatic activity in the extract injected. Thus the extract with the highest enzymatic activity—A^1B^1 308—induced the formation of an antiserum with the highest titer of antibody against tyrosinase (see the examples listed in table 3).

As previously shown, antisera against standard extracts rapidly inactivate and slowly precipitate tyrosinase. Figure 2 illustrates the inactivating effect of antibody to homologous tyrosinase. Within a few seconds after the addition of extract to a mixture of antiserum and substrate, the enzymatic activity is reduced between 50 and 90%. The extent of reduction depends upon the par-

ticular antiserum and extract used and upon the relative concentrations of enzyme, substrate, and antiserum. Previous incubation of the enzyme with an active antiserum before substrate is added effectively reduces initial tyrosinase activity; in some instances the tyrosinase activity is completely destroyed, although generally a small fraction of the activity persists. Cross-reactions between heterologous antisera and an active extract from standard type (fig. 3) show that the activity of an antiserum is not specific for its homologous extract; it reacts equally well with the tyrosinase in other extracts. Antisera to mutants with no tyrosinase activity (A^1B^1 216 in fig. 3) fail to inactivate tyrosinase; such antisera precipitate materials from the crude enzyme extracts but do not affect the tyrosinase. The failure of such extracts to induce the

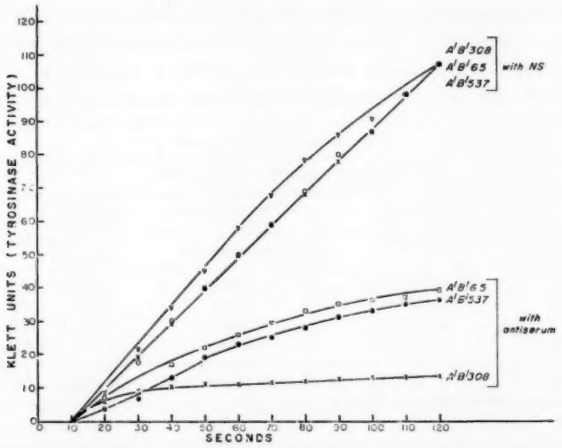

FIGURE 2.—Curve showing the reaction of tyrosinase from three mutants in normal serum and in their antisera. Reaction mixture contained 1 ml serum, 3 ml of buffer, .5 ml dopa, and 1 ml enzyme solution (diluted to make the three mutants approximately equal). Readings were started 10 seconds after mixing.

formation of antibodies against tyrosinase is further evidence that they do not contain tyrosinase which is simply masked by the presence of large molecular inhibitors. Such inhibited tyrosinase molecules if they existed might be expected to function as antigens, at least in the production of precipitating antibodies, even though their enzymatic activity was suppressed. We should note, however, that an enzymatically inactive material related to β-galactosidase in *E. coli* functions as an antigen only in combining with antibodies, not in inducing them (COHN and TORRIANI 1952, 1953).

These quantitative reactions do not critically exclude the possibility that antibodies from different antisera may have some specific differences corresponding to specific differences among the tyrosinase molecules from different mutant extracts. Further tests for specificity were made by means of absorp-

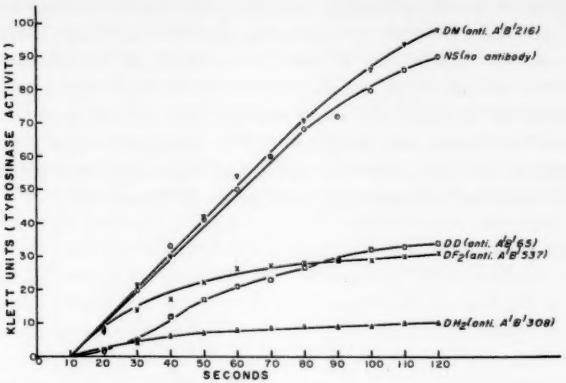

FIGURE 3.—Curves showing the reaction of tyrosinase from standard type in normal serum and in various antisera. Antiserum DM was against a mutant with almost no tyrosinase activity.

tion techniques. Figure 4 shows the results of absorbing active antisera with active extracts and with enzymatically inactive extracts. In table 4 are shown the results of absorbing several different antisera with a variety of enzymatically active extracts. Because the extracts differed in enzymatic activity, the amount of the extract used in the absorption tests was adjusted so that equal total activities for each absorbing extract were employed. It is apparent from table 4 that the heterologous and homologous extracts are equally effective in absorbing antibodies to tyrosinase. It appears, therefore, that the antibodies to tyrosinase in the various antisera do not distinguish among the tyrosinases of different type and mutant cultures. The absorbed antisera are like normal control sera with regard to their effects on tyrosinase.

TABLE 4

Cross-reactions of antisera and tyrosinase from different mutants.

Antiserum	Inducing extract	Tyrosinase activity of injected extract	Absorbing* extract	Remaining antibody activity to tyrosinase
CR_3	S-1	642	S-3	None
			A^1B^165	None
			A^1B^1308	None
			A^1B^1318	None
DD	A^1B^165	1100	S-3	None
			A^1B^165	None
DH	A^1B^1308	6570	A^1B^1308	None
DL	A^1B^1318	3100	S-3	None
			A^1B^1318	None

*At calculated equivalence as discussed in text.

Absorption of active antisera by enzymatically inactive extracts resulted in extensive precipitation but the antibodies against tyrosinase were not precipitated and the supernatant retained its capacity to inactivate tyrosinase. Similarly the antisera made against inactive extracts precipitated many constituents from an enzymatically active extract, but the tyrosinase in the extract was neither inactivated nor precipitated. The enzymatic activity remained in the supernatant and was subject to later inactivation and precipitation on the addition of an antiserum containing antibodies to tyrosinase.

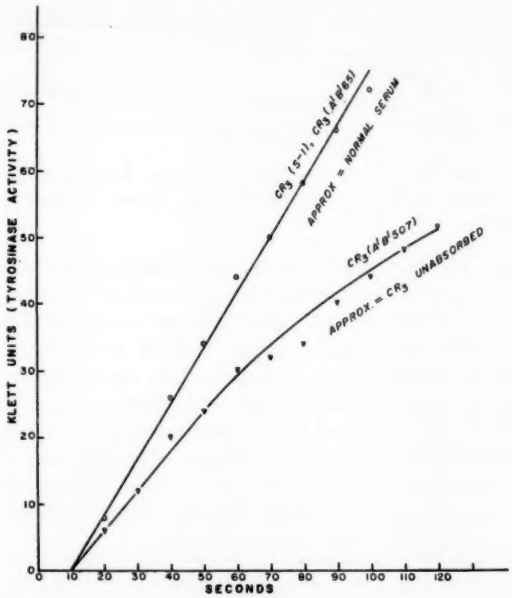

FIGURE 4.—Tyrosinase activity in antiserum CR_3 unabsorbed, and after absorption with extracts from A^1B^1(S-1), $A^1B^1$65, and $A^1B^1$507. Absorption with an active extract renders the antiserum equal to a normal serum, but absorption with an inactive extract does not change the antibody titer to tyrosinase.

The cross-precipitation in absorption experiments demonstrated that the extracts of Glomerella contained antigens common to or similar in all of the extracts. However, the presence of an antigen which will induce antibodies against tyrosinase appears to correspond exactly with the presence of at least a minimum amount of the enzyme activity itself. Similarly, the presence of an antigen which will precipitate the antibodies evoked by the active enzyme depends upon the presence of the enzyme itself. This study therefore provides no evidence of enzymatically inactive materials serologically related to tyrosinase in any of the diverse preparations tested.

This conclusion is further substantiated by the absence of any comple-

mentary inactivation of tyrosinase in mixed antisera. The tyrosinase extracts used in testing for complementary inactivation were from mutants A^1B^1 65, A^1B^1 537 and A^1B^1 308, and from the standard type (extract S-3). Antisera employed were the homologous antisera CR_4 (anti-standard type), DD_2 (anti 65), DF_2 (anti 537), and DH_2 (anti 308). The tyrosinase activities of the extracts and the antibody titers of the antisera were initially quantitatively different. However, by the use of appropriate dilutions it was possible to balance the antibody content of an antiserum against the enzymatic activity of its homologous extract. Then any of the antisera (or their mixtures) at proper dilution could be substituted for one another in inactivating the various enzyme extracts or their mixtures. The different antisera therefore appear to act at the same points in interfering with enzyme activity, and not to summate by acting at independent points. This further implies that there are no serologically recognizable differences among the tyrosinase molecules formed by the various mutant types of Glomerella.

The equivalence points of the various tyrosinase-containing extracts may be determined (OWEN and MARKERT 1954), and a comparison of these equivalence points should serve as a measure of the relative efficiencies of the tyrosinase molecule in the various extracts as an enzyme and as an antigen (table 5). The antigen excess edge of the equivalence zone, at which significant enzymatic activity begins to appear in the supernatants of precipitin tests, was also used to compare the precipitating antibody content of different antisera with reference to several extracts. It was evident that in any particular

TABLE 5

Relative enzymatic and serological activity of tyrosinase in equivalence quantities[1] of extracts as determined for various antisera.

Antiserum designation	Extract designation	Mgs of extract at equivalence	Total tyrosinase activity	Relative tyrosinase activity[2]	Relative serological activity[3]
CR_3	S-3	2.6	2600	1.0	1.0
	$A^1B^1$308	0.4	2600	6.6	1.0
	$A^1B^1$318	0.8	2500	3.1	1.0
DH_2	S-3	6.0	6000	1.0	1.0
	$A^1B^1$308	0.8	5300	6.6	1.1
DL	S-3	1.6	1600	1.0	1.0
	$A^1B^1$318	0.5	1600	3.1	1.0
DD_2	S-3	1.3	1300	1.0	1.0
	$A^1B^1$65	1.3	1400	1.1	0.9
DF_2	S-3	2.4	2400	1.0	1.0
	$A^1B^1$537	2.6	2600	1.0	0.9

[1]The equivalence quantity is the amount of extract required to neutralize the antibodies in 1 ml of antiserum.

[2]Approximate tyrosinase activity per mg of extract compared to extract S-3 which is taken as 1.0.

[3]Antibody-neutralizing activity of the tyrosinase present at equivalence in each extract compared to the antibody-neutralizing activity of the tyrosinase in extract S-3 which is taken as 1.0.

antiserum, the edge of antigen excess appeared at nearly the same number of units of enzymatic activity regardless of which extract was used as a source of the enzyme. The determination of these equivalence points is subject to some error and the differences indicated by the data in table 5 are probably not significant. The absence of striking differences in these equivalence points of different preparations supports the conclusion that the tyrosinase molecules in the different mutant extracts are antigenically identical. The similarity in equivalence points also demonstrates that the activity of tyrosinase molecules is essentially independent of other constitutents of the extract. Variations in enzyme activity in different mutant extracts can therefore be considered an

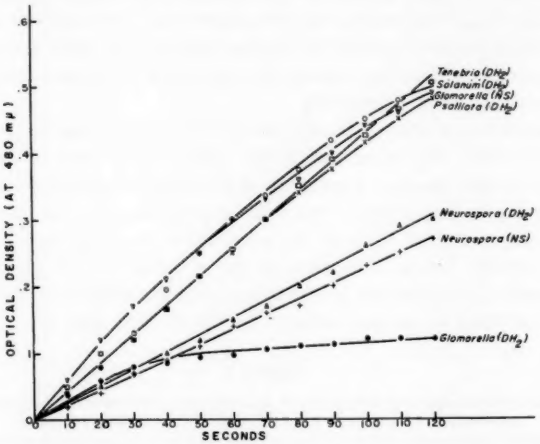

FIGURE 5.—Curves showing the tyrosinase activity of extracts from five species in normal serum and in antiserum to Glomerella tyrosinase. Reactions in normal serum for Psalliota, Solanum and Tenebrio are not plotted since they were almost exactly the same as in antiserum DH_2. Only the Glomerella tyrosinase is drastically inhibited by the antiserum. Readings for the Neurospora tyrosinase were taken at 10-minute intervals and the optical density readings were .1 of the values shown on the graph.

accurate measure of the relative numbers of tyrosinase molecules present in the extracts.

The very rapid inactivation of Glomerella tyrosinase by antibody and the suggested kinetic competition between antibody and substrate dopa (OWEN and MARKERT 1954) strongly imply that the enzymatically active surface is directly involved in the antigen-antibody union. If antigenic and enzymatic surface were identical, then tyrosinase from other species might react with antibodies against Glomerella tyrosinase. Presumably enzyme surfaces are complementary to the substrate surface; since the active enzymatic surface combines with the same substrates in the case of tyrosinases from different species, it might perhaps be expected that these active surfaces would also react with the same antibodies. Accordingly, tyrosinase was extracted from

the fungus Neurospora, the mushroom Psalliota, the potato Solanum, and the mealworm Tenebrio. In addition to the crude extract from Psalliota a commercial, purified preparation with 4,200 catecholase units of tyrosinase activity per ml was used.

The tyrosinase activities of these various preparations were adjusted by dilution with buffer until they were nearly identical in activity. Then a reaction mixture was prepared consisting of 1 ml of 0.02 M DL dopa, 1 ml of dialyzed serum (normal serum, or antisera DH_2 or CR_4) and 1 ml of the enzyme solution. Measurements of dopachrome formation were taken at 10-second intervals in a spectrophotometer (Beckman model B) set at 480 $m\mu$. The Neurospora tyrosinase activity was so low that measurements were taken at 10-minute intervals. The results are illustrated in figure 5. The reactions in normal serum are not graphed for the Psalliota, Solanum or Tenebrio tyrosinases since these reactions were almost identical to the reactions in antiserum. The antisera DH_2 and CR_4 were alike in not affecting tyrosinase activity from non-Glomerella species. The purified commercial Psalliota tyrosinase and the crude extract of Psalliota were equally indifferent to the antibodies against Glomerella tyrosinase, and it is apparent that there is no cross reaction between the antibodies to Glomerella tyrosinase and the tyrosinases from any of the other four species. That antiserum DH_2 is very effective against Glomerella tyrosinase is, however, clearly shown in figure 5.

The availability of antisera containing antibodies that combined with tyrosinase as well as with other antigens found in extracts of Glomerella mycelia prompted an effort to repeat the work of EMERSON (1944) on the induction of mutations by antibodies. Emerson used the fungus Neurospora in his investigations. The conidia of Glomerella, being haploid and uninucleate, provide especially favorable material for measuring the mutagenicity of various agents since the treated spores may be plated out and the mutants evaluated directly without the necessity of first passing through a sexual cross. Accordingly, the conidia of Glomerella were treated with antisera to Glomerella mycelial extracts by the procedures previously reported to induce mutations.

The hypothesis that antibodies may act as mutagens is an attractive one because of the relationship between certain antigens (e.g., blood group antigens) and genes. If a structural relationship exists between the gene and its product—an enzyme or antigen—then the presence of a structurally related antibody at the time of gene replication might conceivably interfere with normal replication in such a way as to produce a gene with an altered structure —that is, a mutation. EMERSON tested this hypothesis by treating *Neurospora* conidia with antisera against culture filtrates, mycelial extracts, or fractions of such extracts. Mutants appeared among the isolates following each type of treatment while no mutants were found among controls. Combining all the data from various types of antiserum treatment EMERSON found 25 mutants out of 695 treated isolates and no mutants out of 276 control isolates.

The present investigations with Glomerella conidia gave quite different

TABLE 6

Treatment of Glomerella conidia with antiserum containing antibodies to tyrosinase.

Treatment	Spore concentration $\times 10^6$/ml	Total isolates	Mutants
67% antiserum CR 24 hrs at 2 °C.	1.2	139	0
67% antiserum CR 24 hrs at 25 °C.	1.2	137	0
67% normal serum 24 hrs at 2 °C.	1.2	94	1
67% normal serum 24 hrs at 25 °C.	1.2	95	0
Untreated controls	1.2	80	0

results. The data are summarized in table 6. It should be noted that neither the normal serum nor the antiserum had any adverse affect on the viability of the conidia. Isolated colonies were evaluated for mutant characteristics by visual inspection only, but this method is sufficient for detecting both morphological and nutritionally-deficient mutants since all nutritional mutants of Glomerella are recognizably different from the standard type even on complete medium. Furthermore, any tyrosinase mutants would have been conspicuous by their altered pigmentation. The results of this experiment, therefore, lend no support to the hypothesis that antibodies are mutagenic. The single mutant found had not been treated with antibodies.

DISCUSSION

These serological studies of tyrosinase are informative with respect to five different but related problems.

(1) Genetic and environmental control of enzyme synthesis.

It has been previously shown (MARKERT 1950) and is again demonstrated here that single gene changes at many different loci alter the tyrosinase activity in extracts from the mycelia of *Glomerella*. At the inception of this investigation three plausible mechanisms were suggested to explain the changed enzymatic activity: (a) gene changes affected the biochemical environment of the tyrosinase molecules in cells and in extracts so that the measurable tyrosinase activity was changed, or (b) gene changes resulted in a qualitative alteration of the enzymatic efficiency of the tyrosinase molecule—that is, different kinds of tyrosinase were produced by different mutants, or (c) gene changes modified the quantity of enzyme synthesized in the Glomerella mycelium. Of these possible explanations, the present study supports the third for the following reasons.

First, the experiments with mixed extracts indicate that there is nothing in the inactive extracts that inhibits active extracts added to them, and nothing in the active extracts that enhances enzymatic activity in inactive extracts

added to them. The differences in tyrosinase activity of these extracts appears to depend on their tyrosinase contents and not on the environments in which tyrosinase functions. Furthermore, the serological studies offer no evidence for inhibitors with antigenic properties comparable to those suggested by DULIÈRE and ADANT (1934) in their study of mealworm tyrosinase.

Second, the tyrosinases of the different extracts appear to be identical as antigens, both in the induction of antibodies and in reactions with antibodies. If the precipitin equivalence tests may be considered to reflect the number of enzyme molecules at equivalence with a given antiserum, then the uniformity of equivalence activity values for various extracts tested against a given antiserum indicates that this " equivalence number of molecules " has virtually identical activity in all of the extracts tested. The differences in activity per mg of extract, therefore, appear to depend on differences in the amount, not in the quality or activity of tyrosinase. The great reduction of tyrosinase activity found in extracts of Glomerella mycelia grown in submerged culture as compared to surface culture is also apparently due to a reduction in the amount of tyrosinase synthesized by the mycelium. The serological characteristics of extracts with very little enzyme activity are the same regardless of whether the reduced enzyme activity is due to genetic or environmental changes. So far, the effects of environment upon the development of enzyme activity have been studied serologically only in the case of submerged and surface cultures. Other environmental effects—temperature, nutrition, etc.—may of course change enzymatic activity by some mechanism other than simple change in the number of enzyme molecules synthesized. It should be noted that HOROWITZ and SHEN (1952) found that Neurospora, when grown on a sulfur-sufficient medium, synthesizes a dialyzable inhibitor of tyrosinase.

(2) Antigenic uniqueness of tyrosinase.

The antigenic properties of tyrosinase appear to be unique. No other antigen present in enzymatically inactive extracts of Glomerella either precipitates antibodies against tyrosinase, or induces antibodies that will inactivate or precipitate tyrosinase. Such inactive extracts do not appear to possess a " precursor " or other protein having a serologically detectable relationship to the finished enzyme molecule. In this regard the Glomerella tyrosinase system appears to be different from the β-galactosidase of *E. coli* studied by COHN and TORRIANI (1952, 1953). These investigators found that an enzymatically inactive protein related, possibly as a precursor, to β-galactosidase would precipitate a large part of the antibody to the active enzyme, although the inactive protein was not itself an effective antigen.

(3) Correpondence of antigenic and enzymatic surface of tyrosinase molecule.

The preceding conclusion may have to be modified if the site of the antigenic configuration of tyrosinase is, in fact, also the site of the enzymatic surface, for then the enzymatic and antigenic properties of the tyrosinase molecule would be inseparable. Three kinds of data suggest the positional

equivalence, at least in part, of these two surfaces. First, the extremely rapid inactivation of tyrosinase by antibody suggests the direct involvement of the enzymatically active surface of the molecule in the antigenic-antibody combination. Secondly, the close correspondence in absorption tests between combining capacity and enzyme activity suggests that these two properties of the extracts simply measure the amount of tyrosinase in the extracts. And thirdly (cf. OWEN and MARKERT 1954), the kinetic competition between substrate and antibody in reacting with the tyrosinase molecule strongly suggests an association between the substrate and the antibody combining sites.

(4) Species specificity of tyrosinase.

Since tyrosinases derived from different species catalyze the oxidation of the same substrates, it seems reasonable to suppose that their enzymatic surfaces are very similar if not identical. However, their antigenic properties are distinctly different—that is, Glomerella tyrosinase is antigenically distinct from Neurospora, Solanum, Psalliota and Tenebrio tyrosinase. Whether the tyrosinases from these last four species are individually distinct has not yet been tested but the presumption is that they too will be found to be distinguishable from one another. This conclusion is supported by the early work of GESSARD (1902a, 1902b) who demonstrated the species specificity of tyrosinase by showing that both cephalopod (cuttlefish) and mushroom tyrosinases were inhibited by homologous antisera, but not by the heterologous antisera. ADAMS (1942) similarly showed that rabbit antiserum against *Psalliota campestris* tyrosinase failed to precipitate the tyrosinase prepared from the related mushroom *Lactarius piperatus*. This species specificity of tyrosinase seems to preclude a simple identity between the antigenic and enzymatic surfaces of tyrosinase.

Studies of the serology of enzymes (cf. SEVAG 1951, for general discussion) have demonstrated that certain enzymes exhibit species specificity in serological tests whereas others show cross-reactions. For example, MILLER *et al.* (1949) demonstrated that antiserum to yeast hexokinase inhibited yeast hexokinase but not rat brain hexokinase. Similarly, KREBS and NAJJAR (1948) produced inhibitory antisera to yeast D-glyceraldehyde 3-phosphate dehydrogenase by injection of the purified yeast enzyme into rabbits and chickens, but such antisera did not cross-react with the equivalent enzyme from rabbit skeletal muscle.

On the other hand HOUSEWRIGHT and HENRY (1947) showed that antipenicillinase produced by injection into rabbits of penicillinase from *B. cereus* inactivated penicillinase from both *B. cereus* and an additional organism, *S. aureus*. HOWE and TREFFERS (1953) found cross-reactions between certain related enzymes (lysine decarboxylase reacted with antisera to arginine decarboxylase) but were able to distinguish serologically between the lysine decarboxylases from three distinct coliform organisms.

In terms of current conceptions of the bases of complementary specificity (cf. PAULING in LANDSTEINER 1945) firm combinations, very slightly reversible if at all, between antibody and enzyme, suggest surface-specific forces

distributed over a relatively large area. In contrast, substrate-enzyme combinations represent a very small area, freely reversible and subject to competitive inhibition by small molecules of appropriate structure. Perhaps the most reasonable explanation of the failure of cross-inactivation of tyrosinase is one that pictures the enzymatic surface as being a part of a larger antigenic surface. The enzymatic surfaces of various tyrosinases could then be identical while species distinctions would be imposed by variations in the remainder of the antigenic surface of the tyrosinase molecule.

(5) Non-mutagenicity of antibodies to tyrosinase.

Several investigators have used antisera in attempts to demonstrate mutagenicity of antibodies, with conflicting results. EMERSON (1944) and ANDERSSON-KOTTÖ (1951) reported positive results using antiserum-treated Neurospora conidia. Unfortunately, in EMERSON's investigation the effects of serum (as contrasted with the antibodies in serum) were not adequately controlled since the control isolates were not treated with normal serum (except for 12 isolates—too few to be significant). In ANDERSSON-KOTTÖ's work the frequency of mutation observed was too small to be more than suggestive. Only 3 mutants were found out of 169 isolates treated at 23°C in 100% antiserum. Treatments at other temperatures and with other spore types yielded negative results. On the other hand FOX and ZIEBUR (1952) reported negative results in their attempt to induce mutation at the inositolless locus in Neurospora by means of antibodies. Their results are of doubtful significance, however, in view of the fact that their serological tests failed to demonstrate antibodies specific for the inositolless locus. In any event neither antiserum nor normal serum were shown to have any mutagenic action at this locus.

In the tests with Glomerella conidia, both normal and antiserum effects were tested, with negative results. The single mutant found after normal serum treatment was consistent with expectations based on the spontaneous mutation rate in Glomerella. The number of spores examined in the Glomerella investigation was fewer than in EMERSON's study, but the frequency of mutation found by EMERSON after antiserum treatment (3.6%) would, if the same frequency held in Glomerella, have yielded about 10 mutants, whereas none was found. This negative result has added significance in view of the ease with which a wider variety of mutants are recognizable in Glomerella as compared to Neurospora. Furthermore, the antiserum was proved to contain antibodies to tyrosinase, and changes affecting this enzyme would have been especially noticeable. Similar negative results were obtained by RYAN *et al.* (1953) who found that antibodies against the enzyme β-galactosidase in *E. coli* were non-mutagenic. Normal γ-globulin was also shown to be non-mutagenic.

SUMMARY

1. The tyrosinase activity extractable from a Glomerella mycelium is dependent upon the genotype of the fungus and upon the environmental conditions under which the mycelium is grown.

2. Crude extracts of Glomerella mycelium are effective antigens when in-

jected into rabbits. When the injected extracts contain an amount of tyrosinase in excess of a rather high threshold, the antisera obtained rapidly inactivate and slowly precipitate tyrosinase from all Glomerella extracts with tyrosinase activity. Extracts with little or no tyrosinase activity (from mutants or submerged cultures) fail to induce antibodies against tyrosinase.

3. Genetic or environmentally induced changes in the tyrosinase activity of a mycelium are related to changes in the number of tyrosinase molecules synthesized by the mycelium rather than to changes in the nature of the tyrosinase molecule. This point is supported by several lines of evidence including the fact that the amount of tyrosinase activity found in an extract closely parallels the ability of the extract to combine with antibodies against tyrosinase.

4. The enzymatically active surface of the Glomerella tyrosinase molecule appears to be a part of the antigenically active surface.

5. The antigenic surfaces of tyrosinase molecules are species specific since no cross reactions were found between antibodies to Glomerella tyrosinase and tyrosinase from Psalliota, Solanum, Neurospora, and Tenebrio.

6. Antisera containing antibodies to Glomerella tyrosinase are not mutagenic when applied to Glomerella conidia.

ACKNOWLEDGMENT

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LITERATURE CITED

ADAMS, M. H., 1942 The reaction between the enzyme tryosinase and its specific antibody. J. Exp. Med. **76:** 175–184.

ANDERSSON-KOTTÖ, IRMA, 1951 Mutations in antiserum treated Neurospora. Hereditas **37:** 289–290.

COHN, M., and A. M. TORRIANI, 1952 Immunochemical studies with the β-galactosidase and structurally related proteins of *Escherichia coli*. J. Immunol. **69:** 471–491.

1953 The relationships in biosynthesis of the β-galactosidase and Pz-proteins in *Escherichia coli*. Biochem. et Biophys. Acta **10:** 280–289.

DULIÈRE, W. L., and M. C. ADANT, 1934 A study of the immunological action of tyrosinase from mealworms. Biochem. J. **28:** 1659–1664.

EMERSON, S., 1944 The induction of mutations by antibodies. Proc. Nat. Acad. Sci. **30:** 179–183.

FOX, A. S., and N. K. ZIEBUR, 1952 An attempt to induce specific back-mutation at the inositolless locus in *Neurospora crassa* by means of antibodies. Genetics **37:** 581–582.

GESSARD, C., 1902a Tyrosinase animale. C. R. Soc. Biol., Paris **54:** 1304–1306.

1902b Antityrosinase animale. C. R. Soc. Biol., Paris **54:** 1398–1399.

HOROWITZ, N. H., and MARGUERITE FLING, 1953 Genetic determination of tyrosinase thermostability in Neurospora. Genetics **38:** 360–374.

HOROWITZ, N. H., and SAN-CHIUN SHEN, 1952 Neurospora tyrosinase. J. Biol. Chem. **197:** 513–520.

HOUSEWRIGHT, R. D., and R. J. HENRY, 1947 Studies on penicillinase. III. The effect of antipenicillinase on penicillin-resistant organisms. J. Bact. **53:** 241–247.

HOWE, A. F., and H. P. TREFFERS, 1953 Precipitation and inhibition of bacterial amino acid decarboxylases by rabbit antibody. Federation Proc. **12:** 447.

KREBS, E. G., and V. A. NAJJAR, 1948 The inhibition of *d*-glyceraldehyde 3-phosphate dehydrogenase by specific antiserum. J. Exp. Med. **88**: 569–577.

MARKERT, C. L., 1950 The effects of genetic changes on tyrosinase activity in Glomerella. Genetics **35**: 60–75.

1952 Radiation-induced nutritional and morphological mutants of Glomerella. Genetics **37**: 339–352.

MILLER, R. E., V. Z. PASTERNAK and M. G. SEVAG, 1949 Inhibition of yeast hexokinase by homologous antiserum. J. Bact. **58**: 621–625.

OWEN, R. D., and C. L. MARKERT, 1954 Effects of antisera on tyrosinase in Glomerella extracts. J. Immunol. (in press).

PAULING, L., 1945 Molecular structure and intermolecular forces. In Landsteiner, K., The specificity of serological reactions, rev. ed., C. C. Thomas, Springfield, Ill., 1945, pp. 276-293.

RYAN, F. J., PHYLLIS FRIED and ELSA GONZALEZ, 1953 On the inability of antibodies to induce mutations in bacteria. Am. Nat. **87**: 383–387.

SEVAG, M. G., 1951 Immuno-catalysis and related fields of bacteriology and biochemistry. Rev. 2nd ed. C. C. Thomas, Springfield, Ill.

SUSSMAN, A. S., and C. L. MARKERT, 1953 The development of tyrosinase and cytochrome oxidase activity in mutants of *Glomerella cingulata*. Arch. of Biochem. Biophys. **45**: 31–40.

WHEELER, H. E., and J. W. MCGAHEN, 1952 Genetics of Glomerella. X. Genes affecting sexual reproduction. Amer. J. Bot. **39**: 110–119.

EVIDENCE AGAINST A STRAIGHT END-TO-END ALIGNMENT OF CHROMOSOMES IN DROSOPHILA SPERMATOZOA[1]

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IN order to understand the response of the hereditary material in the mature spermatozoa of *Drosophila melanogaster* to physical and chemical mutagens, it is necessary to know the form and arrangement of the chromosomes in the sperm head. For, depending on the plan of its organization, one would expect different relative frequencies of point mutations and gross chromosome alterations after a given mutagenic treatment.

The view that the chromosomes are arranged end to end in a straight line along the long axis of the sperm head has been based on cytological observations. GOWEN and GAY (1933), working with Drosophila, measured the sizes both of the sperm head and of the individual chromosomes during oögonial metaphase. The close correspondence between the length of the sperm head (approximately $7\frac{1}{4}\mu$) and the total length of a haploid set of metaphase chromosomes containing either the X or Y chromosome (approximately 7μ), together with the close similarity in chromosome and sperm-chromatin widths, led them to conclude that the chromosomes are arranged end to end (presumably in a straight line). WOLF (1939) reported similar observations.

Recently, COOPER (1952) reported that the chromosomes in the nearly mature sperm head of Drosophila can be seen in certain fixed preparations. In one case, where a complete set of chromosomes could be recognized, they resembled ordinary mitotic metaphase chromosomes in sizes, shapes, and proportions, and were arranged in single file along the long axis of the sperm head. In general, however, according to figures and information kindly furnished us by DR. COOPER after we had sent him a copy of the present paper as originally submitted, the chromatin of the nearly mature spermatozoa had the appearance not of a straight line but rather of a coarse helix with more or less side-by-side arrangement of its several coils, and these when squeezed apart were seen to be made up of chromosomes of metaphase appearance—observations which we regard as not inconsistent with our own data and interpretations here presented. RAPOPORT (1941), in a paper (dealing primarily with another subject) which has come to our attention only after the completion of our present work, makes the following statement concerning

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Drosophila spermatozoa: " we examined four kinds of preparations stained after Feulgen of the following objects: normal sperm cells, sperm cells without a sex-chromosome, with an extra sex-chromosome and, finally, with a closed X-chromosome. Neither data in favour of a linear end to end arrangement, nor in favour of the existence of a fixed localisation order of the chromosomes (especially in closed X) in the sperm cells were found." Unfortunately, no data or other details were included in his paper.

The view that sperm-head chromosomes do not have a straight end-to-end arrangement but as a rule overlap each other has seemed to us to be indicated by genetic evidence. After irradiating sperm with fast neutrons, MULLER and VALENCIA (1951) found that the frequency of translocations varied linearly with dose even at doses sufficient to produce several proton tracks per sperm. MULLER (1954a) interpreted this to mean that those broken ends which underwent union with one another were derived from breaks caused by the same proton track, and that breaks therefore had to be very close together before there was a good chance of union between their broken ends. This implied that the non-homologous chromosomes which underwent mutual translocation of large pieces (the usual type of translocation) overlapped each other very considerably in the sperm head at the time of irradiation. Recent experiments on the incidence of translocations and sex-linked recessive lethal mutations after treatment of spermatozoa with two doses of neutrons, reported in papers by MULLER now in press (1954b) and in preparation, confirm the earlier data and interpretation.

In order to obtain additional cytological evidence concerning the arrangement of the chromosomes in the sperm head, determinations were made of the length of the chromatin mass in individual spermatozoa containing different chromosome assemblages. Two stocks of *D. melanogaster* were used, an ordinary wild-type stock, Oregon-R, and a special stock made available by DR. E. NOVITSKI and described by LINDSLEY and NOVITSKI (1950). Whereas one-half of the sperm from Oregon-R males contain an X chromosome and one-half a Y, half the sperm produced in NOVITSKI's stock contain neither the X nor the Y while the other half carry both, attached together, and with a part of the heterochromatic material even further duplicated. The autosomal content, however, is quantitatively identical in all these sperm.

EXPERIMENTAL PROCEDURE

To obtain and prepare sperm heads for measurement, the seminal receptacle was dissected from a fertilized female into a small drop of saline (prepared according to BUCK and MELLAND 1942) which was then quickly flooded with the usual aceto-orcein fixative-stain and covered with a coverslip. A current of fresh stain was kept flowing beneath the coverslip for at least ten minutes, after which the excess stain was removed and the coverslip margins sealed with white Karo syrup. To avoid, as much as possible, distortion due to compression, enough aceto-orcein was left beneath the coverslip and sufficient Karo was applied so that the receptacle was barely held in position be-

tween slide and coverslip. Slides were usually used 2–4 hours after preparation, at which time the sperm heads showed the most contrast with their surroundings; there was little precipitation of stain around the receptacle and none within it, and the Karo had not diffused into this region. Such preparations of sperm are especially adequate for this type of study since the heads are distributed over a considerable length of the receptacle, usually lying parallel to the long axis of the tube, and are often separated from each other, straight, and almost completely in one focal plane. Tails of sperm are unstained so that only the chromatin of their heads is readily visible. Beginning at the blind end of the receptacle and proceeding to its opening into the vagina, measurements were made of all suitable sperm on each slide. Sets of measurements from slides of the two stocks were taken alternately, whenever possible, by means of a camera lucida, using a green Corning glass filter and compensating ocular (15×) and apochromatic objective (90×, N.A.1.40) lenses. The magnification at table-level was about 1850 diameters.

THE RESULTS AND THEIR FORMULATION

Seven slides of Oregon-R sperm and thirteen slides of sperm from NOVITSKI's stock were examined, and 244 and 322 measurements of the length of the chromatin mass were made, respectively. The individual determinations from each of these slides are shown in figure 1, as well as the mean values for each slide. It may be noted that there is considerable variation in these means both in the Oregon-R and the NOVITSKI stock. A statistical analysis has shown that this variation of means is several times as great as would be expected as a result of random sampling if the sperm on the different slides of the same stock had represented the same material. This interslide variability must be due, in large measure, to differences in fixation and in compression of the sperm heads in different receptacles. The mean lengths of chromatin mass for the Oregon-R and NOVITSKI stocks are, at table level magnification, 18.3 and 17.6 mm, respectively. The difference between these means is, however, without significance, because of the wide, determinate variation between the means of the different slides of each stock.

In addition to this variation which exists in the actual length of the fixed chromatin masses on different slides, there are five sources of variation resulting from the measurement process. These depend on the depth of focus of the optical system, the straightness of the sperm head, the degree to which it is horizontal, the marking of the limits of length seen using the camera lucida, and the reading of these limits with a ruler. The first three of these sources of error are considered to have been largely randomized, and probably do not often have an error exceeding 1 mm per chromatin mass at table-level magnification. There may not have been so random an error in the marking and reading of the lengths. For example, it may be noticed that in the NOVITSKI stock (figure 1) there are 10 measurements at 16.0 mm but only one at 16.1. This type of error is on an exceedingly small scale, however, and would not affect the results from different slides, still less those from different stocks,

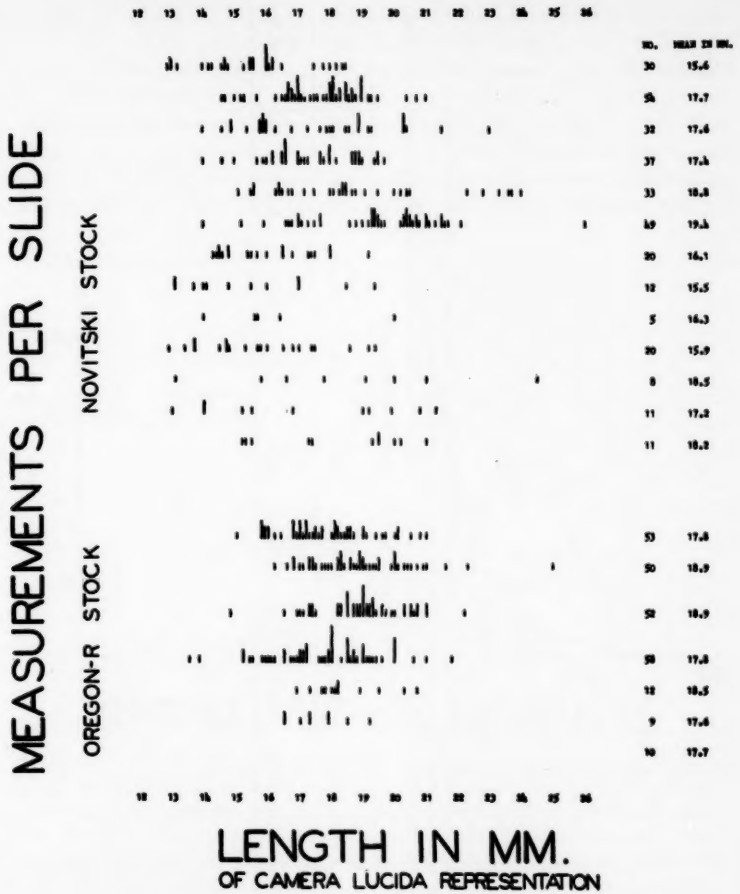

FIGURE 1.—The individual camera lucida lengths of 244 sperm chromatin masses from the Oregon-R stock and 322 from NOVITSKI's stock are arranged according to slide. For each stock, slides measured later are placed above those measured earlier.

in a differential manner to any significant extent. Moreover, all these five errors of measurement are insignificant in relation to table-readings of the order of half a millimeter for indivdual sperm or of 0.2 millimeters for averages.

Since the high interslide variation, caused by differences in technique of preparation, greatly increases the dispersion and tends to invalidate comparisons of the distributions in the two stocks, a procedure was employed which eliminated this variation. The first step was to calculate the mean value for

TABLE 1

Distribution of measurements of the length of chromatin in sperm heads containing different numbers of chromosomes. (Length expressed in terms of percentage of mean length of sperm on the given slide.)

Stock	No. chromatin masses measured	Percent of sperm having lengths designated												
		70.1–75.0	75.1–80.0	80.1–85.0	85.1–90.0	90.1–95.0	95.1–100.0	100.1–105.0	105.1–110.0	110.1–115.0	115.1–120.0	120.1–125.0	125.1–130.0	130.1–135.0
Novitski	275	0.4	0.7	6.9	9.1	14.6	18.2	18.9	17.1	5.5	5.1	1.8	1.1	0.7
Oregon-R	213	0.0	1.4	0.5	10.3	13.2	24.4	24.9	14.6	7.1	2.8	0.5	0.0	0.5

each slide on which 20 or more measurements had been made. This comprised 4 slides of the Ore-R and 8 of the NOVITSKI stock, including 275 and 213 measurements, respectively. Slides with fewer measurements were omitted in order to avoid introducing an appreciable error caused by the statistical unreliability of these means. It was calculated that for the groups of 20 or more this error would be so small as to have a negligible influence on the results sought. All the individual measurements were then expressed as percentage deviations from the mean value for their slide, and all the measurements of a

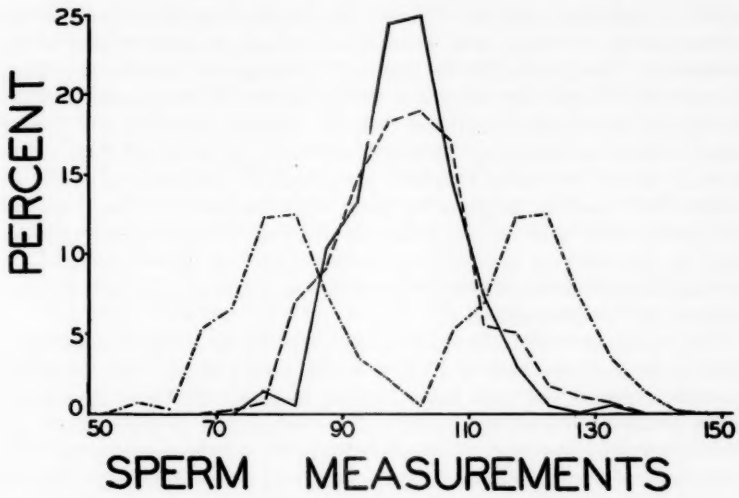

FIGURE 2.—The graphs shown are those for the Oregon-R (continuous line) and the NOVITSKI (dashed line) stock, drawn from the values of table 1, and the one expected for the latter stock (dot-dash line) on the hypothesis of a straight-line end-to-end arrangement in the sperm head of chromosomes proportioned like those at metaphase. Sperm frequencies of each stock are expressed in terms of percent of measurements lying within each 5% interval to right and left of mean of slide from which the given measurement was taken. (Only slides of 20 or more measurements were used.)

given stock, thus expressed, were then combined into one distribution, for which the values of the standard deviation and of the quartiles were calculated.

In table 1 the results for each stock are grouped into class-intervals corresponding to each difference of 5% from the mean sperm length, which is taken as 100%. Figure 2 represents the same results as a graph for each stock. In both table and figure the frequencies are expressed in terms of the percentage of total observations made on the stock in question which fell within each 5% interval. In making the figure these observed percentage frequencies were shown as dots in the middle of their respective 5% intervals of sperm length, the ordinate representing the frequency and the abscissa the length. These dots, for each stock, were then connected by a line. It is evident that

both distributions are effectively unimodal; but the NOVITSKI stock appears to have a greater dispersion.

On further analysis of the distributions, that for Oregon-R proves to have a standard deviation of 8.21 ± 0.40% of its mean. The calculation has been based on the observed measurements when these were grouped in classes having an interval of only 1% instead of the 5% used in the table and figure. The error of the determination has been based on the assumption of a normal distribution. That the Oregon-R distribution is approximately a normal one, as expected of a random sample of items whose deviations have been determined by numerous small factors which vary in large measure independently of one another, is indicated by a number of measured characteristics of the distribution. Among these are the positions of median and quartiles in relation to mean and standard deviation, and the proportion of observations with deviations, in each direction, greater than the standard deviation and greater than 1.5 times and twice the standard deviation, respectively. All these values agree as closely with those calculated for a normal distribution as would be expected for a random sample of the given size taken from an actually normal distribution. This does not yet prove the distribution to be entirely normal, however, for much the same fit might be obtained if the curve represented a combination of two simlar normal curves having means only a short distance apart, as will be discussed later.

The data for the NOVITSKI stock, subjected to the same type of calculation, show a standard deviation of 10.07 ± 0.43% of the mean. Here again the standard deviation and mean have the same positions relative to the median and quartiles as in a normal curve, and the frequencies of observations exceeding 1.5 times and twice the standard deviation are also not very far from those expected for a normal curve having the given standard deviation. On the whole, however, the frequencies in the more extreme regions tend to be higher, in relation to the standard deviation, than for the Oregon-R curve, though this difference in shapes is not enough to be convincing in itself. What is clearly significant in a comparison of the two curves is the difference in their degree of dispersion, as measured by either their standard deviations or quartiles. Thus the difference in their standard deviations, being 1.86 ± 0.59, is 3.2 times its own standard error, and had a chance of occurrence, by random sampling, of less than 1 in 1000 (in this "expected" direction), if the sperm of the two stocks had really possessed the same dispersion.

INTERPRETATION

If the reality of the difference in dispersion between the distributions of sperm lengths for the two stocks be admitted, it follows with high probability that the heterochromatin of the sex chromosomes, or more specifically, the heterochromatic blocks (MULLER and GERSHENSON 1935), have an appreciable size, relative to the euchromatin, as they do in mitotic chromosomes, although they are not necessarily as large as in the latter. For if the blocks and the heterochromatin of the sex chromosomes in general were as minute, rela-

tively to the rest of the chromatin, as they are in salivary and other interphase stages, no appreciable difference in the size of sperm would be expected from their addition or subtraction. Hence the sperm of the NOVITSKI stock would possess no wider dimorphism, and would show no greater dispersion, than those of the Oregon-R stock, in view of the fact that the sperm of these two stocks differ only in respect to the distribution of their sex-chromosome heterochromatin.

In the attack on the problem of whether the arrangement of the chromosomes in the spermatozoa is a straight end-to-end one, let us first assume, as a working hypothesis, that the blocks, and therefore the heterochromatin, are about as large, relatively to the rest of the chromatin, as they are at mitosis. In that case, the amount of chromatin in a sperm with an X would be sensibly equal to that in a sperm with a Y; hence the Oregon-R stock would have no quantitative sperm dimorphism. But in the NOVITSKI stock, the amount of chromatin in the sperm with X and Y attached would bear a ratio to that in the sperm lacking a sex chromosome lying somewhere between 7:4 and 6:4 (i.e., 3:2). Moreover, if the chromosomes were arranged in straight single-file this would also be the ratio of the means of the sperm-head lengths of the two classes of sperm.

The figure 7:4 comes nearer the apparent value as shown in most figures, in which the Y is approximately as long as the X and each of them is about 1½ times as large as one of the arms of the major autosomes. However, the objects are so small that the error, even of the relative sizes, is very large. Because of stretching, the error of relative salivary chromosome lengths is also large. There is reason, based on the lower frequency of induced lethal mutations in the X than in the arms of the major autosomes, to suspect that the euchromatic portion of the X may be not more than 0.8 of the size of the euchromatic portion of an arm of a major autosome. The proximal heterochromatin of the X at mitosis is not more than half as long as the euchromatin of the X, and since it is more or less tapering it might become packed down, in the sperm, to occupy less than half the length of the euchromatin. It is also to be taken into consideration that the heterochromatin blocks of the major autosomes occupy an appreciable space at mitosis (being in total about ¼ as long as the euchromatin of these autosomes) and that those of the small fourth chromosome are probably as long or longer than the euchromatin of that chromosome. Taking all these items into consideration at once (but not the fact that the attached X-plus-Y has somewhat more mitotic heterochromatin than a normal X and a normal Y added together) we arrive at the minimum value of 3:2 for the ratio of lengths of the two classes of sperm of the NOVITSKI stock, on the assumptions of heterochromatin blocks as large as at mitosis and lengthwise, end-to-end arrangement of straight chromosomes.

The question next arises, what would be the distribution of sperm lengths on these assumptions when, to be conservative, the ratio of 3:2 is used in our calculations? Since, on the present assumptions, the Oregon-R sperm is quantitatively homogeneous in its chromatin content, we may use its distri-

bution to represent that of each of the component distributions of the NOVITSKI stock sperm. However, the means of these two components will be symmetrically situated about the Oregon-R mean, with a distance between them equal to 40% of the Oregon-R mean (since on the scale of 3:2 for the NOVITSKI means the Oregon-R mean would be 2.5, and the difference $3-2$, or 1, is 40% of 2.5). Hence to construct our artificial curve we first construct two curves, each having the same spread and shape as the Oregon-R curve but only half its height at each point, and place them with their means respectively at 80% and 120% as compared with the Oregon-R mean. We then combine them into a single curve by adding their heights wherever they overlap with one another, and finally join the ordinates thus established by a continuous line. In this way the curve has been constructed which is shown as a dot-dash line in figure 2. Its standard deviation may be calculated to be $\sqrt{20^2+8.2^2}$, or $\pm 21.6(\%)$.

It is immediately apparent that this hypothetical curve, with its extreme bimodality, is quite irreconcilable with the observed curve for the NOVITSKI stock. Moreover, the difference between the enormous standard deviation of the hypothetical curve and that ($10.07 \pm 0.43\%$) observed for the NOVITSKI stock is many times its own statistical (standard) error. It must therefore be concluded either that the sperm chromosomes are in straight end-to-end alignment, or that their heterochromatin blocks are substantially smaller than at mitosis, or that both our previous assumptions are wrong at once.

Let us see if we can save the assumption of straight end-to-end arrangement by assuming the heterochromatin blocks to be relatively smaller than at mitosis. We may first try out the most extreme assumption regarding them, namely, that they are of negligible size as in interphase and salivary chromosomes. Here, however, we come up against two objections. First, as earlier noted, the significantly greater dispersion of the NOVITSKI than of the Oregon-R stock distribution shows that the heterochromatin blocks are of appreciable size. Second, if the blocks were inappreciable there would be a considerable dimorphism among the Oregon-R sperm. On the assumption that the euchromatin in the X is only 0.8 as long as in the arms of the major autosomes, the two classes of sperm would have means approximately 91.5% and 108.5% of the mean of the mixed lot of sperm. Thus, even if all sperm of a given class were exactly equal in length, as measured, the standard deviation of the mixed lot would be 8.5%, since all sperm would have this amount of deviation from the common mean. The resultant "curve" would then consist of two vertical lines separated by an interval of 17%. But only $8.2 \pm 0.4\%$ has been observed as the standard deviation, so that nothing is left for deviation about the means of the component distributions. Moreover, as the shape of the observed curve shows, much (if not all) of its dispersion must in fact be caused by differences of real or apparent length among sperm of the same sex-chromosome content. Hence the premise of straight end-to-end arrangement of chromosomes completely lacking blocks is ruled out.

A little calculation and plotting of graphs was then carried out to ascertain

how much dimorphism the Oregon-R stock might conceal. The procedure followed was based on the principle that the standard deviation-squared of a distribution resulting from a combination of two distributions with the same dispersions, but means differing by d, is equal to d^2 plus the standard deviation-squared of the component distributions. This procedure showed that if the Oregon-R distribution is a combination of two equal component distributions of identical shape, the means of these components cannot differ by more than 8 or 10% (i.e., they will stand at not more than $\pm$ 4%, or possibly $\pm$ 5%, from the mean of the mixed distribution). For if they differ more than this they will result either in too sharply bimodal a combined curve to be reconciled with the observed Oregon-R curve, or else in a curve having a standard deviation significantly greater than that observed for the Oregon-R sperm.

If, now, we take the extreme assumption of a 10% difference in means for Oregon-R, we arrive at a contradiction with observation when we calculate the result for the NOVITSKI stock. To calculate the NOVITSKI stock curve, we must first determine the difference in means which its two components would have on the given assumptions. This is derived as follows. The difference in means in the Oregon-R stock if the blocks had been negligible would, as noted previously, have been at least 17%, but on the present assumptions it is at most 10%. Therefore in this case a differential effect on the length of at least 7%, acting in an opposite direction to the 17% euchromatically caused difference, is being exerted by heterochromatin. It is exerted by the heterochromatin-block *excess* carried by the Y as compared with the X, and this is approximately one half of all the heterochromatin-block material carried by both the X and Y, considered together (since that in the Y is to that in the X approximately as 3 : 1, the excess of Y over X thus being $3-1$, or 2, while the total is $3+1$, or 4, which is double the excess, 2). That is, the total sex-chromosome heterochromatin would occupy a length of twice 7%, or 14%, of the mean sperm length. (This is approximately half of its observed length at mitosis.) Hence, in the NOVITSKI stock sperm, in which the mean of one component differs from that of the other both by reason of the 17% difference caused by the euchromatin of the X, and also of the 14% minimum difference just calculated to arise from the blocks, the means would differ by 31% from each other, and would accordingly occupy places at $\pm$ 15.5% from the mean of the whole lot of sperm. The separation of means, then, is only moderately less in this case than that shown in the hypothetical bimodal curve of figure 2.

Yet despite this wide separation between the two means the dispersion about each of them has become less than in the curve of figure 2. For the present assumptions, according to which the Oregon-R curve has two components, with means differing by $\pm$5% from the common mean, have reduced the residual variability allowed to each of these components to a standard deviation of 6.5%, in order to meet at the same time the condition that the standard deviation of the total remain only 8.2% (by the calculation

that, approximately, $8.2 = \sqrt{(6.5)^2 + 5^2}$). Now, when the dispersion of each component of the NOVITSKI-stock curve is reduced to this standard deviation of 6.5%, the bimodality is sharpened and becomes even more pronounced than that in the hypothetical curve of figure 2, and is even less reconcilable than before with the observed NOVITSKI stock curve of figure 2. At the same time, the standard deviation of the hypothetical curve becomes $\sqrt{6.5^2 + 15.5^2}$, or $\pm$ 16.8, instead of the observed 10.07 $\pm$ 0.43 or the value of 21.6 calculated on the assumption of heterochromatin like that at mitosis.

We have now examined the effects, on the hypothetical NOVITSKI-stock distribution, of supposing the total sex-chromosome heterochromatin to have the least size consistent with the Oregon-R data, namely, 14% of all the chromatin, and at the other extreme, a size as great as in mitotic chromosomes, namely, 27% or more. We have seen that in both cases, so long as a straight end-to-end arrangement is assumed, the hypothetical curve is glaringly at variance with the observed one. Any assumption regarding heterochromatin sizes intermediate between these extremes must give a similarly impossible result. This will be better realized in consideration of the following course of reasoning. It has just been seen that the assumption of a minimum of sex-chromosome heterochromatin (14%) results in a curve with far too marked a bimodality and too great a standard deviation to fit the observed results for the NOVITSKI stock. If, now, the total heterochromatin of the sex-chromosomes of the sperm is assumed to be any greater than this, by an amount termed *h*, this amount *h* will be added to the amount of separation between the means of the two component curves of the hypothetical NOVITSKI stock which has just been calculated, thus *increasing* its standard deviation to a corresponding (and almost equal) extent. At the same time, the dimorphism in the Oregon-R stock will be reduced only by $\frac{h}{2}$, since in that stock only half of this heterochromatin constitutes the excess of Y over X, and the dispersion allowed the component curves will only be slightly increased thereby, so that the bimodality will remain marked. Hence there are no allowable assumptions regarding heterochromatin size in the sperm whereby a straight end-to-end arrangement can give a distribution even distantly reconcilable with that observed for the NOVITSKI stock.

Having thus abandoned the hypothesis of a straight end-to-end arrangement, let us see whether there is some other hypothesis which will fit the data adequately. In choosing it, we should be guided by the information, provided by the significantly greater dispersion found for the NOVITSKI stock than for the Oregon-R stock, that the heterochromatin blocks remain developed to a perceptible degree in the spermatozoon stage. This being the case, the simplest assumption is to consider them as being of the same size, relative to the euchromatin, as they are at mitosis, a premise which also agrees best with the observations of COOPER (1952) on the chromosomes of not quite mature spermatozoa. Adopting this assumption, and also taking, as before, the figure 3:2, i.e., 1.5, as the ratio between the bulks of the two classes of sperm of

the NOVITSKI stock, it is evident that the ratio of their lengths would be $\sqrt[3]{1.5}$, or 1.145, if the sperm of both classes had the same shape and therefore had lengths proportionate to the cube root of their volumes. This would give, as the mean lengths of the two classes, $1.0725 \pm .0725$, or, on the scale which takes the mean of the combined distribution as 100%, it would give $100 \pm 6.75\%$. To get the standard deviation of the combined distribution, we must take into account not only this but also the standard deviation of the component curves, $\pm 8.21\%$, obtained from the Oregon-R results. We then arrive at $\sqrt{6.75^2 + 8.21^2}\%$, or 10.6%, as the resultant standard deviation. This agrees excellently with the observed value of $10.07 \pm 0.43\%$ for the standard deviation of the NOVITSKI stock, being removed from it by only 1.2 times the standard error of the latter value, and by less than the error of the difference itself.

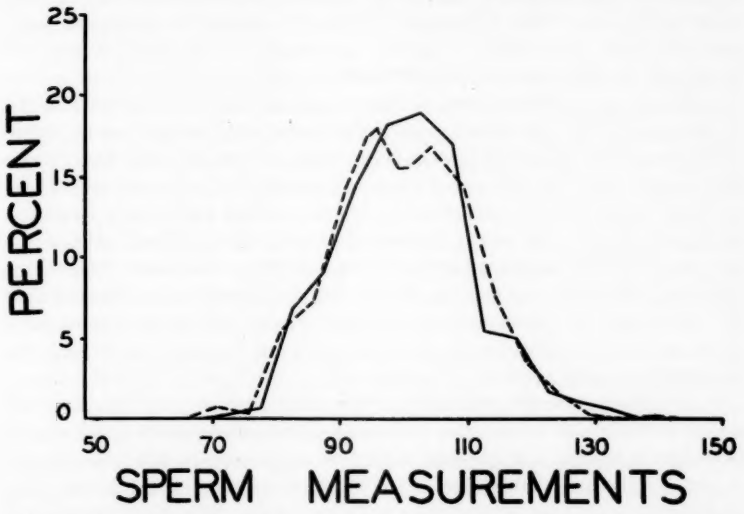

FIGURE 3.—Superposition of observed NOVITSKI stock graph (continuous line) with hypothetical graph (dashed line) based on two combined graphs of same shape as that of Oregon-R but with means related as $\sqrt[3]{3/2}$.

In order to compare the shape of the resulting curve with that observed for the NOVITSKI stock, we have plotted the two together in figure 3. The hypothetical (dashed-line) curve of this figure was constructed in the same way as the hypothetical curve of figure 2, except that in the present case the means of the two component curves by the summation of which it was built were taken as being in the positions $100 \pm 6.75\%$, in accordance with the above calculation. It is to be observed that the hypothetical and observational curves fit each other very satisfactorily throughout, when the errors of sampling to which they are subject are taken into consideration.

Despite the good fit of the calculated to the observed values, the correct-

ness of the premises on which the calculations were based cannot yet be considered as actually proved, but only made probable. It might still be held that the lengths of sperm do not tend to be proportional to the cube root of their volume. In that case, however, the agreement of theory with observation would have to be ascribed in part at least to coincidence. It would have to be supposed that technical errors and/or complicating influences acting on the length of sperm heads had so counterbalanced the effects of the operation of a rule different from that of proportionality of lengths to the cube root of volumes (a rule according to which the shapes of sperm heads of different sizes do *not* tend to remain constant) as to mask the main principle at work. It would be idle to discuss these other possibilities in detail here. However, attention may be called to the possibility of an adaptive mechanism which tended to hold sperm heads to a given, functionally advantageous length, even in the face of differences in their chromatin content.

Light on the questions here at issue might be thrown by further studies of the same type as the present ones, to determine whether their results agreed with those here presented, especially in cases in which other chromosome differences than those here studied were concerned. In the meantime, however, the lack of discrepancy between our observations and the calculated consequences of our assumptions, considered in connection with the simplicity of these assumptions, argues strongly for the validity of the latter. And, in any case, the hypothesis of a straight end-to-end arrangement of chromosomes in the sperm head of *Drosophila melanogaster* can no longer be considered as admissible for the majority of spermatozoa, even though, conceivably, that arrangement might occur in occasional cells.

If, as seems probable, the rule of the proportionality of the chromatin length in the sperm to the cube root of the chromatin mass is valid, even if only approximately, the question arises, in what way is this rule brought about? As previously mentioned, the rule would imply that the sperm heads of different sizes have substantially the same shape. This would have to be true despite the fact that when the chromosome contents of the sperm of different sizes were laid out in any fixed arrangement (such as end-to-end in one row or in two rows, etc.), and then subjected to any proportionate deformation, the resulting chromatin masses would *not* have lengths proportional to the cube roots of these masses. The production of this relationship is more likely to follow, in case the chromosomes involved are of mitotic shapes to start with, if their mutually repulsive forces were reduced to a minimum or overcome, and they were pressed into a compact mass of a given shape, perhaps more or less ovoid, as though they were so much modelling clay, and if after that they became squeezed out into a very elongated shape of fairly constant proportions. In that case there might not be a substantial change in the arrangements of the parts with regard to one another, aside from this lengthwise deformation, and as a result there might be considerable overlapping of the chromosomes and of their parts in most of the sperm. Indeed,

to explain the results with neutrons previously referred to, taken together with the occasional production of highly complex mutual ("cyclical") interchanges of parts, several different chromosomes, and chromosome arms, must not infrequently be present in the same cross-section of a sperm head.

It is of course conceivable that the chromosomes of the mature sperm, instead of being, primarily, in more or less discrete masses as at mitosis, have a conformation resembling, in respect to interpenetration of parts (but not in respect to amount of non-chromatin material), that of interphase. However, the evidence derived from our present results, indicating the existence of blocks at this stage, as well as the cytological observations earlier referred to, make it more likely that the chromosomes are more like those of mitosis, except in regard to their crowding and deformation.

SUMMARY

1. The lengths of the chromatin masses were measured in spermatozoa of a normal stock and in those of a stock (NOVITSKI's attached X.Y) in which half of them contained both X and Y and the rest contained neither. The frequency distribution of lengths had a significantly greater dispersion in the latter stock than in the former.

2. It is inferred that this difference in distribution of lengths was caused by the presence in the spermatozoa of heterochromatin blocks, similar to those in mitotic chromosomes, and that these blocks were differently distributed in the two stocks among their two classes of spermatozoa.

3. The relatively small dispersion and the effective unimodality of distribution of chromatin-lengths found in both stocks are facts irreconcilable with the hypothesis of a straight end-to-end arrangement of chromosomes in the sperm head of *Drosophila melanogaster*.

4. When the chromosomes of the spermatozoa are assumed to have the same proportions of parts, including blocks, as those of mitotic and meiotic divisions, and when they are assumed to become squeezed together into masses of substantially the same shape in the sperm head, regardless of the amount of chromatin present, so that the lengths of these masses are proportional to the cube roots of their volumes, the resulting distributions of lengths, calculated for the two stocks, are found to be in excellent statistical agreement with the distributions observed by us.

5. It is to be expected that a method of formation of sperm heads of the type above suggested would entail considerable overlapping of chromosomes and of chromosome parts, along the length of the sperm heads. This type of arrangement had been inferred earlier, on the basis of genetic data concerning the relations between neutron dosage and the frequency of induced translocations. If, on the contrary, a straight single-file arrangement of chromosomes had been established for the sperm head, a radical revision of current theories regarding the way in which structural changes of chromosomes are produced would have been required.

LITERATURE CITED

BUCK, J. B., and A. M. MELLAND, 1942 Methods for isolating, collecting and orienting salivary gland chromosomes for diffraction analysis. J. Hered. **33:** 173–184.

COOPER, K. W., 1952 Studies on spermatogenesis in *Drosophila*. Yearb. Philos. Soc., 1951: 146–147.

GOWEN, J. W., and E. H. GAY, 1933 Gene number, kind, and size in *Drosophila*. Genetics **18:** 1–31.

LINDSLEY, D. L., JR., and E. NOVITSKI, 1950 The synthesis of an attached XY chromosome. Drosophila Information Service **24:** 84.

MULLER, H. J., 1954a The manner of production of mutations by radiation. Vol. 1, Chapter VIII: 475–626, Radiation Biology, ed. A. Hollaender. McGraw-Hill: N. Y.
1954b The relation of neutron dose to chromosome changes and point mutations in Drosophila. I. Translocations. Amer. Nat. **88** (in press).

MULLER, H. J., and S. GERSHENSON, 1935 Inert regions of chromosomes as the temporary products of individual genes. Proc. Nat. Acad. Sci. **21:** 69–75.

MULLER, H. J., and J. I. VALENCIA, 1951 The localization of the mutagenic action of neutron-induced ionizations in Drosophila. (Abstr.) Rec. Genet. Soc. Amer. **20:** 115–116; and Genetics **36:** 567–568.

RAPOPORT, J. A., 1941 Mutations restituting the telomere. C. R. (Dokl.) Acad. Sci. U.R.S.S., N.S. **31:** 266–269.

WOLF, E., 1939 Die Anordnung der Chromosomen in Spermienkern von *Dicranomyia trinotata* Meig. Chromosoma **1:** 336–342.

THE EFFECTS OF OXYGEN ON THE FREQUENCY OF X-RAY-INDUCED MUTATIONS IN HABROBRACON EGGS[1]

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AS long ago as 1921 HOLTHUSEN observed an effect of anoxia on X-rayed cells. Interest in this factor and its relation to radiation damage has recently been revived. BACQ (1951) has reviewed the earlier work and latest developments have been described by HOLLAENDER, BAKER and ANDERSON (1951), HOLLAENDER (1952) and GILES (1952). One group of investigators has been concerned with the question as to whether the reduction in X-ray-induced chromosome injury correlated with reduced oxygen concentration is due to a reduction in primary breaks (breakage hypothesis) or to an increase in restitution of primary breaks (reunion hypothesis). The settlement of this question would aid in understanding the mechanism whereby ionizing radiations produce changes in chromosome structure. Interesting and important facts have been discovered but the question as to the cause of the "oxygen effect" has not been settled. The work on Habrobracon eggs herein reported was undertaken in order to determine if some evidence for one theory or the other could be secured. No proof for either theory has been found but, in the opinion of the author, the facts favor the breakage hypothesis.

MATERIAL AND METHODS

The technique used is that devised originally by the author (1940, 1945b) and developed by HEIDENTHAL (1952, 1953). Wild-type stock females were used in all experiments. When mated, they were outcrossed to ivory-eyed males. Control hatchability ranged from 94 to 98 percent. Unmated females produce sons only, all haploid; mated females lay about one-third unfertilized eggs which give rise to haploid males and about two-thirds fertilized which develop into diploid females. In occasional experiments the female ratio is greatly increased for reasons not understood at present.

Females were isolated as virgin and fed for three days on a generous supply of host caterpillars. They were then removed and kept for at least 16 hours at 30°C without hosts, in the absence of which they do not oviposit but store

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in uterine sacs up to twenty eggs, all in first meiotic metaphase (metaphase I). They were then divided into two groups, one irradiated in air, the other in nitrogen. After irradiation each group was subdivided and only one part mated. Mass matings were made and males were left with females for one hour. Practically all females mate during this time.

Each female was then placed in a small Stender dish with one host caterpillar. Dishes were kept in an incubator at 30°C for six hours. Exhaustive tests have demonstrated that under these conditions only those eggs irradiated in metaphase I are laid. At the end of this six-hour period females were discarded, a record taken of eggs laid, and dishes returned to incubator. Forty-two hours later, numbers of larvae and of unhatched eggs were recorded, and hosts with larvae attached were placed in shell vials for continuation of development to adulthood. F_1 females were isolated as virgin and a record taken of sex ratio for each experiment. This is important as a check on estimated dominant lethal rates.

F_1 females were handled as were their mothers for hatchability studies except that they were transferred at intervals to fresh caterpillars until large enough numbers of eggs were obtained to be of significance as tests for recessive lethal or visible mutations.

Throughout this paper the term "lethal mutation" is used to designate embryo lethals. Other types will be specifically described.

Hatchability of eggs laid by unmated X-rayed females gives an accurate measure of freedom from *all* lethal changes affecting the embryos (haploid). Hatchability of eggs laid by females mated after irradiation gives a measure of freedom from *all* embryo lethals in sons (haploid), of freedom from *dominant* embryo lethals in daughters (diploid). Surviving daughters, unmated, lay eggs all or almost all of which hatch if no recessive lethals have been induced in eggs from which they have developed. If one recessive lethal has been induced in an egg, the female developing from it, when unmated, will lay eggs, one-half of which hatch. If two nonlinked recessive lethals have been induced, one-fourth will hatch, etc. Postembryo recessive lethals were observed as larvae, pupae, or unenclosed adults constituting about 50 percent of total progeny and similar in appearance and in stage at death for any one fraternity.

For exposure to X-rays in atmospheres of nitrogen and of air, females were chilled slightly in order to induce temporary inactivation and placed in small lucite tubes closed at the ends with perforated caps. Rubber tubing was attached to each end of these tubes. The gases were conducted from tanks and bubbled through containers of water after passing over the females. Rate of bubbling was kept relatively constant. For 1100 r, females in nitrogen and in air were exposed simultaneously. Gases used were commercially prepared water-pumped nitrogen and compressed air and they were turned on and off at the same time as were the X-rays. Females in nitrogen were flushed with air immediately after irradiation.

In view of the observations of GILES and RILEY (1950) who found that anoxia during irradiation was the effective condition, and of evidence that

long exposures to nitrogen alone were reducing hatchability of Habrobracon eggs, females were exposed to nitrogen only during irradiation in experiments summarized herein. Air was used as a control since GILES and RILEY (1950), BAKER and EDINGTON (1952), and KENWORTHY (1953) have observed that increase in effect of oxygen concentration is gradual after a rapid rise to a percentage approaching that in air. An exposure to nitrogen alone for three minutes and twenty seconds, the maximum length of X-ray treatments used, did not affect hatchability.

The X-rays were administered by means of a Maxitron machine operating at 250 kvp and 30 ma with a 3-mm aluminum filter added to the inherent filtration of the tube. Dosage measurements were made before each exposure with a Victoreen 250-r thimble chamber inserted within a dummy lucite tube placed in the same position as those within which the wasps were irradiated. At the target distance used (39 cm) dosage rate varied from 330 to 340 r/minute during the several weeks of the experiment. Exposures of 1100 and 500 r were used with time as the variable.

In all, 49,405 eggs were observed for hatchability.

RESULTS

In table 1A are summarized the hatchability percentages of eggs laid by the females X-rayed in nitrogen and in air. These data are used to estimate dominant lethal rates of embryos given in table 1B. The facts involved in this derivation are outlined in table 2, from which it should be obvious that, in Habrobracon, mutations in each chromosome behave as do those in the X chromosome of Drosophila.

TABLE 1

A. F_1 Hatchability data of eggs X-rayed in air and in nitrogen: a. laid by unmated females; b. laid by mated females.

B. Percentages of dominant embryo lethals estimated from F_1 hatchability data.

C. Percentages of viable F_1 females heterozygous for recessive mutations, derived from F_2 data: a. embryo lethals; b. postembryo lethals; c. visibles.

D. Number of recessive embryo lethal mutations per F_1 female tested, derived from F_2 data.

	Dose of X rays (r)						
	1100					500	
	Air		Nitrogen		Diff. ± $\sigma_{diff.}$	Air	
A							
a.	89/704	12.64%	352/644	54.65%	42.01 ± 2.33	268/483	55.49%
b.	180/866	20.78%	923/1511	61.08%	40.30 ± 1.84	651/1058	61.53%
B	433/577	75.04%	360/1006	35.78%	39.26	248/705	35.18%
C							
a.	21/138	15.21%	32/620	5.16%	10.05 ± 3.18	18/312	5.76%
b.	5/123	4.06%	13/653	1.99%	2.07 ± 1.86	10/312	3.20%
c.	9/125	7.20%	15/653	2.29%	4.91 ± 2.38	8/312	2.56%
D	28/138	0.20%	42/620	0.07%	0.13 ± 0.03	23/312	0.07%

TABLE 2

Embryo lethal mutations in eggs of females X-rayed with 1100 r in air and in nitrogen.

Unfertilized	Fertilized
All eggs from unmated ♀♀ and 33% from mated ♀♀	66% of eggs from mated ♀♀
Die	*Die*
Dominants	Dominants/+
Dominants and recessives	Dominants and recessives/+
Recessives	
Survive	*Survive*
No lethals	Recessives/+
12.64% in air	No lethals (+/+)
54.65% in nitrogen	24.96% in air
	64.22% in nitrogen

Dominant lethal percentages are calculated by multiplying one-third of total eggs laid by mated females by hatchability percentage of eggs from unmated females. This gives the number of males to be expected among survivors from mated females and, therefore, the expected number of females among survivors. Expected female survivors divided by two-thirds of total eggs laid by mated females equals percentage without dominant lethals. Sex ratios of actual survivors from mated females check well with expectation based on these estimates.

Recessive mutation rates are given in table 1C. They are derived from records of eggs and of progeny of unmated F_1 females. Postembryo lethal rates are least dependable because of possibilities of environmental causes of death. Since some F_1 females are heterozygous for more than one recessive lethal, the number of recessive embryo lethals per viable F_1 female can be computed. This is given for females of each group in table 1D.

Postembryo dominant lethals were not recorded in the present experiment. Their study would involve special care in ensuring that each F_1 larva has an environment adequate for survival since each lethal is represented by only one individual, in contrast to F_2 tests for recessive lethals in which as large a number as is desired can be obtained for each lethal. Procedure for estimating percentages of dominant postembryo lethals would be the same as that outlined for dominant embryo lethals.

Data for 1100 r were obtained first. When the striking differences in response between progenies of females X-rayed in nitrogen and in air were observed, it was decided to determine a dose in air which would give F_1 results comparable to those obtained for 1100 r in nitrogen. This dose proved to be 500 r, and subsequent study of the F_2 data showed no significant differences from mutation rates induced by 1100 r in nitrogen (table 1). The conclusion seems evident that all types of chromosome change induced by X-rays in metaphase I eggs respond similarly to absence of oxygen at time of irradiation.

DISCUSSION

The haploid chromosome complement of Habrobracon consists of five V's, four J's, and one rod. When exposed to X-rays in metaphase I (described as anaphase I by some authors) the ten tetrad chromosomes lie on the spindle without contact with each other. The shorter ones have terminalized so that only the ends of separating dyads remain in contact. Longer ones still have one subterminal chiasma per long arm. Parts of chromosomes between chiasmata and centromeres (proximal regions) appear thinner than those distal to chiasmata and suggest that tension is being exerted on the former.

Cytological study of later stages after irradiation in metaphase I (WHITING 1945a, 1945b) discloses fragments between daughter nuclei in telophase I but no bridges. These fragments are relatively large and their double nature can sometimes be recognized. They increase in number with increase in dose. Bridges in one or both spindles in telophase II coupled with the presence of these fragments in division I, and the one-hit type of hatchability curve for dominant lethals (HEIDENTHAL 1953), support the conclusion that single events can break adjoining chromatids and that lateral fusion of broken ends follows. Bridges continue to be formed in subsequent cleavage divisions when a chromatid with a terminal deletion has reached the egg pronucleus.

The absence of bridges in division I following irradiation indicates that all permanent breaks involving two chromatids are proximal to chiasmata or that lateral fusion does not take place if two chromatids are broken by a single event distal to a chiasma.

SAX and MATHER (1939) and SAX (1942) showed that permanent X-ray-induced breaks in Tradescantia chromosomes are not at random but are more numerous in proximal regions of chromosome arms. They attributed this to the role of the centromere in controlling mechanical stresses in the coiled chromonemata. LEWITSKY and SIZOVA (1935), who found a similar concentration of X-ray-induced breaks in chromosomes of Crepis, suggest that differential susceptibility may be caused by greater stretching at the proximal end (quoted by SAX and MATHER). This was eliminated as an explanation by these authors for the prophase chromosomes which they were studying but it might apply to late meiotic metaphase where an obvious thinness is observable in the proximal region. DARLINGTON (1937) writes concerning first meiotic division, " It is evident that pairs of chromatids resist separation, for bivalents which have the greatest length of chromatids to pull apart lag behind the rest. . . . The simplest and quickest separation is found amongst bivalents with terminal chiasmata." In some cases " the parts of the chromosomes between the centromeres and the terminal chiasma are so small as to be drawn into a fine thread which may be invisible."

The observations in connection with irradiation of metaphase I chromosomes and those of the authors just quoted suggest that tension may be responsible for the high sensitivity of metaphase I eggs to X-rays, and that most or all double breaks induced in proximal arms are permanent. The chance of a

broken chromatid being included in the egg pronucleus under these conditions is one in two.

Changes other than terminal deletions cannot be studied cytologically in Habrobracon because of the small size of the chromosomes and the absence of giant chromosomes such as are found in many Diptera. The fact that tetrads are not in contact with each other at time of irradiation suggests that translocations would not occur. There remain inversions, repeats, and interstitial deletions which, in view of the nature of the curves (HEIDENTHAL 1953), must be one-hit and therefore small for the most part.

In Sciara, by salivary analysis following irradiation of eggs in metaphase I, REYNOLDS (1941) found interstitial deletions and inversions only. In a similar study BOZEMAN and METZ (1949) found repeats, inversions, interstitial deletions, and one translocation. How many of these aberrations would be lethal in the haploid but not in the diploid is a question—interstitial deletions almost certainly and some of the others undoubtedly.

In view of the findings outlined it appears that changes involving one chromatid only are responsible for most, if not all, recessive lethals and that these must result from reunion after breakage since terminal losses would have a dominant lethal effect. The relative infrequency of recessive lethals when compared with dominant lethal rates can be explained (1) by the possibility that some of the changes induced in single chromatids are nonlethal, (2) by the dominant lethal effect of terminal deletions, and (3) by the fact that the chance of a changed chromatid being included in the egg pronucleus is only one in four in contrast to double breaks (dominant lethals) where the chance is one in two. There remains also the possibility that, because of the synapsed condition of the chromatids, X-ray-induced single "hits" tend to break both chromatids much more frequently than single ones. Recessive changes must be due to the reunion of injured chromatids while dominant lethals obviously are not. This suggests that there may be less tension when a single chromatid is broken than when two are broken at the same point.

If these suggestions concerning the relation of X-ray-induced chromosome changes in Habrobracon tetrads to tension apply, it follows that the chances of the occurrence of recessive lethals will be the same in centric or in acentric synapsed chromatids. Incidence of each class of lethal, then, will be independent of the other.

Because of the differences in rates and in apparent causes of dominant and recessive mutations it was felt that this study might throw some light on the nature of the oxygen effect. KENWORTHY (1953) had shown frequencies of X-ray-induced lethals in Habrobracon eggs to be strikingly affected by different concentrations of oxygen.

Relevant conclusions of other authors are summarized as follows: GILES and RILEY (1950) have demonstrated that the presence of oxygen during the actual period of exposure to X-rays rather than before or after is essential and point out that this favors the breakage hypothesis. RILEY, GILES and BEATTY (1951) conclude that average restitution time for broken chromosome ends is the same in the presence or in the absence of oxygen. This likewise supports

the breakage hypothesis. SCHWARTZ (1952) favors the reunion hypothesis and bases his conclusion on the differential protection of hypoxia against interstitial and terminal deletions in maize. GAULDEN, NIX and MOSHMAN (1953) conclude that "The fact that neuroblasts do not respond to change in oxygen tension (at irradiation) in the same way as to a change in dose of radiation suggests that the influence of oxygen tension on the response of these cells to radiation involves more than merely a change in the amount of injury produced by the radiation."

On the basis of the facts and hypotheses concerning cytological changes induced by X-rays in metaphase I chromosomes in Habrobracon and of the criteria of the authors just quoted for judging evidence as related to one hypothesis or the other, the following conclusions have been drawn.

If breakage is the only condition that changes during irradiation in reduced oxygen, dominant and recessive lethals should change at the same rate.

If reunion is the only condition that changes and tension is a factor in the high incidence of dominant lethals, these should remain constant under all degrees of oxygen concentration whereas recessive lethals might be expected to change.

There remains the possibility that in reduced oxygen, in this case in nitrogen, tension itself is reduced for, according to GAULDEN, CARLSON and TIPTON (1949), within a few seconds after pure nitrogen was introduced into hanging-drop preparations of living neuroblasts, the spindles of those in metaphase were either partially or completely destroyed. When the chorion was left intact on embryos this drastic effect was not observed. Metaphase I eggs in the present study had intact chorions which were obviously penetrated by the nitrogen since the effect on X-ray-induced injury of only slightly over three minutes of exposure was striking but hatchability of controls exposed only to nitrogen for this period was normal. If tension were reduced by a change in the spindle, dominant lethals should decrease more rapidly than recessives on either the breakage or reunion hypothesis.

SUMMARY

Metaphase I eggs of Habrobracon were exposed to X-rays in atmospheres of air and of nitrogen, the latter administered only during the period of irradiation. Dominant embryo lethals, recessive embryo and postembryo lethals, and visible mutations were recorded. The fact that all types were reduced at the some relative frequency in nitrogen, 500 r in air producing almost identical percentages of mutations as 1100 r in nitrogen, afford, in the opinion of the author, some evidence in favor of the conclusion that reduction in oxygen concentration reduces primary breaks and that other factors influencing final conditions are unchanged in the irradiated cell.

LITERATURE CITED

BACQ, Z. M., 1951 L'action indirecte du rayonnement X et ultraviolet. Experientia **7**: 11–19.

BAKER, W. K., and C. W. EDINGTON, 1952 The induction of translocations and recessive lethals in Drosophila under various oxygen concentrations. Genetics **37**: 665–677.

BOZEMAN, M. L., and C. W. METZ, 1949 Further studies on sensitivity of chromosomes to irradiation at different meiotic stages in oöcytes of Sciara. Genetics **34**: 285–314.

DARLINGTON, C. D., 1937 Recent advances in cytology. Philadelphia, P. Blakiston's Son and Company, Inc.

GAULDEN, M. E., J. G. CARLSON and S. R. TIPTON, 1949 Effects of carbon dioxide and nitrogen gas on mitosis as observed in living neuroblasts of grasshopper embryo. Anat. Rec. **105**: 496–497.

GAULDEN, M. E., M. NIX and J. MOSHMAN, 1953 Effects of oxygen concentration on X-ray-induced mitotic inhibition in living Chortophaga neuroblasts. J. Cell. Comp. Physiol. **41**: 451–470.

GILES, N. H., JR., 1952 Recent evidence on the mechanism of chromosome aberration production by ionizing radiations. Symposium on Radiobiology, Oberlin College, New York, John Wiley & Sons, Inc.

GILES, N. H., JR., and H. P. RILEY, 1950 Studies on the mechanism of the oxygen effect on the radiosensitivity of Tradescantia chromosomes. Proc. Nat. Acad. Sci. **36**: 337–344.

HEIDENTHAL, G., 1952 X-ray induced recessive lethals in Habrobracon. Genetics **37**: 590. 1953 A comparison of X-ray induced dominant and recessive lethals in first meiotic metaphase eggs and in sperm of Habrobracon. Genetics **38**: 668.

HOLLAENDER, A., 1952 Physical and chemical factors modifying the sensitivity of cells to high-energy and ultraviolet radiation. Symposium on Radiobiology, Oberlin College. New York, John Wiley & Sons, Inc.

HOLLAENDER, A., W. K. BAKER, and E. H. ANDERSON, 1951 Effect of oxygen tension and certain chemicals on the X-ray sensitivity of mutation production and survival. Cold Spring Harbor Symp. Quant. Biol. **16**: 315–325.

HOLTHUSEN, H., 1921 Beiträge zu Biologie der Strahlenwirkung. Pflüger's Arch. Ges. Physiol. **187**: 1–24.

KENWORTHY, W., 1953 The effect of oxygen concentration on the induction of lethality and chromosomal aberrations by X-rays in Habrobracon and Sciara. Ph.D. Dissertation, University of Pennsylvania, 56 pp.

LEWITSKZ, G., and M. SIZOVA, 1934 Further studies on regularities in chromosome transformations in *Crepis capillaris* induced by X-rays. C. R. Acad. Sci. U.R.S.S. **5**: 86–87.

REYNOLDS, J. P., 1941 X-ray induced chromosome rearrangements in the females of Sciara. Proc. Nat. Acad. Sci. **27**: 204–208.

RILEY, H. P., N. H. GILES, JR. and A. V. BEATTY, 1951 The oxygen effect on X-ray induced chromatid aberrations in Tradescantia microspores. Genetics **36**: 572–573.

SAX, K., 1942 The distribution of X-ray induced chromosomal aberrations. Proc. Nat. Acad. Sci. **28**: 229–233.

SAX, K., and K. MATHER, 1939 An X-ray analysis of progressive chromosome splitting. J. Genet. **37**: 483–490.

SCHWARTZ, D., 1952 The effect of oxygen concentration on X-ray-induced chromosome breakage in maize. Proc. Nat. Acad. Sci. **38**: 490–494.

WHITING, A R., 1940 Sensitivity to X-rays of different meiotic stages in unlaid eggs of Habrobracon. J. Exp. Zool. **83**: 249–269.

1945a Dominant lethality and correlated chromosome effects in Habrobracon eggs X-rayed in diplotene and in late metaphase I. Biol. Bull. **89**: 61–71.

1945b Effects of X-rays on hatchability and on chromosomes of Habrobracon eggs treated in first meiotic prophase and metaphase. Amer. Nat. **79**: 193–227.

AN EXTENSION OF THE CONCEPT OF PARTITIONING HEREDITARY VARIANCE FOR ANALYSIS OF COVARIANCES AMONG RELATIVES WHEN EPISTASIS IS PRESENT[1]*

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IT is convenient, for purposes of description and analysis, to consider the phenotypic expression of a characteristic as a sum of an hereditary or genotypic value and of an environmental value. If the actual joint results deviate from this linear description (i.e., if interaction effects exist) the breeder or geneticist must exercise caution in extrapolating from his results because in this case the hereditary and environmental values are defined specifically in terms of each other (NELDER 1950). For some characteristics a transformation of scale may help in coming closer to additivity (WRIGHT 1950).

With this linear description, the total or phenotypic variance may be considered to consist of the hereditary, environmental and interaction variances, and also of covariance terms if the components are correlated in their occurrence. The covariance between heredities and environments is often a troublesome feature in human and livestock populations. For example, where dairy cattle are fed in proportion to their production, the better genotypes are provided better environments. However, in designed experiments correlation in occurrence can, for the most part, be avoided by randomization devices.

FISHER (1918) partitioned the phenotypic variance further by subdividing the hereditary variance into an additive portion resulting from average effects of genes, a portion resulting from dominance effects (allelic interactions) of genes and a portion resulting from epistatic effects (non-allelic interactions) of genes. FISHER showed the distribution of the additive and dominance portions in correlations between various relatives in a randomly mating population. The present paper shows the subdivision of the epistatic variance into components and gives the distribution of these epistatic components in the covariances or correlations between relatives.

PARTITIONING THE HEREDITARY VARIANCE

The partitioning of the hereditary variance of diploid organisms that have no multiple alleles can be illustrated by considering two loci, each with two

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alleles (A, a and B, b). If the coupling and repulsion double heterozygotes are identical phenotypically, 9 genetic types, as in table 1, are possible. The Y's and f's in table 1 are the hereditary values and relative frequencies of the indicated types, respectively. The hereditary values are actually phenotypic values averaged over all other loci and environments. The subscripts are related to the loci and to the number of genes present. A dot (.) indicates a marginal frequency or mean.

If frequencies at one locus are uncorrelated with frequencies at another locus (algebraically, all $f_{ij} = f_{i.}f_{.j}$) the total variance among the Y's in table 1 can be partitioned exactly into a marginal variance for the A locus (i.e., variance among the row means in table 1), a marginal variance for the B locus, and a joint or interaction variance. Furthermore, each of the marginal variances can be partitioned into a linear and quadratic variance; and correspondingly, the interaction variances can be broken up into four components which are linear

TABLE 1

Hereditary values and relative frequencies of the nine genetic types for two loci, each with two alleles.

AABB	*AABb*	*AAbb*	*AA* −
Y_{22}	Y_{21}	Y_{20}	$Y_{2.}$
f_{22}	f_{21}	f_{20}	$f_{2.}$
AaBB	*AaBb*	*Aabb*	*Aa* −
Y_{12}	Y_{11}	Y_{10}	$Y_{1.}$
f_{12}	f_{11}	f_{10}	$f_{1.}$
aaBB	*aaBb*	*aabb*	*aa* −
Y_{02}	Y_{01}	Y_{00}	$Y_{0.}$
f_{02}	f_{01}	f_{11}	$f_{0.}$
−*BB*	−*Bb*	−*bb*	
$Y_{.2}$	$Y_{.1}$	$Y_{.0}$	$Y_{..}$
$f_{.2}$	$f_{.1}$	$f_{.0}$	$f_{..} = 1$

by linear, linear by quadratic, quadratic by linear, and quadratic by quadratic. This linear and quadratic treatment of a 3×3 factorial, where each of the partitions corresponds to one of the eight degrees of freedom, is found in statistical textbooks. The loci are the factors and each factor has three levels represented by the three combinations that can occur with two alleles. In genetic terminology additive and dominance are used in place of linear and quadratic, respectively.

What is to be shown is equally true in the presence of linkage so long as the frequencies at different loci are uncorrelated. If frequencies are correlated, which they would be under phenotypic assortative mating, the following partitioning does not hold. Other causes of correlated frequencies are discussed by LUSH (1948) under the subject of disequilibrium.

Partitioning the variance will be illustrated by using the eight orthogonal scales in table 2. These orthogonal scales serve the same purpose as orthogonal comparisons or polynomials (SNEDECOR 1946) in computing a portion of the total variance for each degree of freedom. The only new feature here is that the

TABLE 2

Hereditary values, frequencies, and eight orthogonal scales which are utilized in partitioning the hereditary variance.

Scale	Genetic type								
	AABB	*AABb*	*AAbb*	*AaBB*	*AaBb*	*Aabb*	*aaBB*	*aaBb*	*aabb*
Y	Y_{22}	Y_{21}	Y_{20}	Y_{12}	Y_{11}	Y_{10}	Y_{02}	Y_{01}	Y_{00}
f	f_{22}	f_{21}	f_{20}	f_{12}	f_{11}	f_{10}	f_{02}	f_{01}	f_{00}
W_1	$2v$*	$2v$	$2v$	$v-u$	$v-u$	$v-u$	$-2u$	$-2u$	$-2u$
W_2	$1/f_{2.}$	$1/f_{2.}$	$1/f_{2.}$	$-2/f_{1.}$	$-2/f_{1.}$	$-2/f_{1.}$	$1/f_{0.}$	$1/f_{0.}$	$1/f_{0.}$
W_3	$2y$	$y-x$	$-2x$	$2y$	$y-x$	$-2x$	$2y$	$y-x$	$-2x$
W_4	$1/f_{.2}$	$-2/f_{.1}$	$1/f_{.0}$	$1/f_{.2}$	$-2/f_{.1}$	$1/f_{.0}$	$1/f_{.2}$	$-2/f_{.1}$	$1/f_{.0}$
W_5	$4vy$	$2v(y-x)$	$-4vx$	$2y(v-u)$	$(v-u)(y-x)$	$-2x(v-u)$	$-4uy$	$-2u(y-x)$	$4ux$
W_6	$2v/f_{.2}$	$-4v/f_{.1}$	$2v/f_{.0}$	$(v-u)/f_{.2}$	$-2(v-u)/f_{.1}$	$(v-u)/f_{.0}$	$-2u/f_{.2}$	$4u/f_{.1}$	$-2u/f_{.0}$
W_7	$2y/f_{2.}$	$(y-x)/f_{2.}$	$-2x/f_{2.}$	$-4y/f_{1.}$	$-2(y-x)/f_{1.}$	$4x/f_{1.}$	$2y/f_{0.}$	$(y-x)f_{0.}$	$-2x/f_{0.}$
W_8	$1/f_{22}$	$-2/f_{21}$	$1/f_{20}$	$-2/f_{12}$	$4/f_{11}$	$-2/f_{10}$	$1/f_{02}$	$-2/f_{01}$	$1/f_{00}$

*$u = f_{2.} + f_{1.}/2$, $v = 1-u$, $x = f_{.2} + f_{.1}/2$, $y = 1-x$.

joint frequencies are not equal to each other but are proportional to the marginal frequencies. The requirements of the orthogonal scales are:

$$(1)\ \sum_{i,j} f_{ij} W_{tij} = 0, \quad \text{and} \quad (2)\ \sum_{i,j} f_{ij} W_{tij} W_{t'ij} = 0.$$

The first requirement insures that deviations around the mean are compared. The second requirement insures that the comparisons are orthogonal, which means simply that they are uncorrelated. The eight scales or partitions of the variance are one for each of the eight separate degrees of freedom in a 3×3 table. The symbols u, v, x and y in table 2 are the frequencies of the genes *A*, *a*, *B* and *b*, respectively

The partition of the variance, σ_t^2, corresponding to any particular scale, W_t, is found in the following manner:

$$\sigma_t^2 = (\sum_{i,j} f_{ij} Y_{ij} W_{tij})^2 / \sum_{i,j} f_{ij} W_{tij}^2,$$

which, in statistical terminology, is

$$\sigma_t^2 = (\text{Cov } YW_t)^2 / \sigma_{W_t}^2 = \beta_{YW_t}^2 \sigma_{W_t}^2 = \rho_{YW_t}^2 \sigma_Y^2,$$

where Cov, β and ρ are covariance, regression coefficient and correlation coefficient, respectively. The t^{th} partition of the variance is the variance due to regression on the t^{th} orthogonal scale.

This particular set of scales (among the many others mathematically possible) was chosen for its utility. The scales pertaining to the marginal comparisons of each locus were chosen to separate the marginal variance into the same additive (linear) and dominance (quadratic) portions that were long ago shown to be useful for expressing simply the correlation between parent and offspring and between other relatives. The other four scales, which relate to the interactions among the loci, also permit expressing simply the correlations among the interaction effects of one relative and those of another relative. This is the primary purpose of introducing the orthogonal scales.

The first two scales, W_1 and W_2, are concerned only with the means for the rows in table 1 and thus only with the marginal variance for the *A* locus. For example, the means and frequencies for the *A* locus are:

Genetic type	*AA*	*Aa*	*aa*
Mean	$Y_{2.}$	$Y_{1.}$	$Y_{0.}$
Frequency	$f_{2.}$	$f_{1.}$	$f_{0.}$
W_1	2v	v-u	-2u
W_2	$1/f_{2.}$	$-2/f_{1.}$	$1/f_{0.}$

The marginal variance of Y for the *A* locus is broken into two parts, one part being the variance due to the regression of the marginal means on the linear scale designated as W_1, and the other part being the variance due to deviations from this regression. The variance due to regression,

$$\sigma_1^2 = 2uv\left(\frac{f_{2.}}{u} + \frac{f_{0.}}{v}\right)\left[\frac{\frac{f_{2.}}{u}(Y_{2.} - Y_{1.}) + \frac{f_{0.}}{v}(Y_{1.} - Y_{0.})}{\frac{f_{2.}}{u} + \frac{f_{0.}}{v}}\right]^2$$

is the additive variance for the A locus and the variance due to deviations from regression on the linear scale,

$$\sigma_2^2 = \frac{f_{2.}\, f_{1.}\, f_{0.}}{4uv - f_{1.}}[(Y_{2.} - Y_{1.}) - (Y_{1.} - Y_{0.})]^2,$$

is the dominance variance for the *A* locus. This latter variance is also the variance due to regression on the scale designated as W_2.

The additive variance is proportional to the square of the average effect, β_{YW_1}, of the genes; this average effect being a weighted average of the two effects or differences $Y_{2.} - Y_{1.}$ and $Y_{1.} - Y_{0.}$ corresponding to the comparisons $AA - Aa$ and $Aa - aa$. Each difference represents an effect of replacing *a* by *A*, $A - a$, but in each case the effect is measured in the presence of a different allele. If the two differences are not exactly the same, i.e., the effect of replacing *a* by *A* changes according to whether *a* or *A* is present, then the alleles at this locus interact, and the interaction is reflected in the dominance variance, σ_2^2.

In a similar manner σ_3^2 and σ_4^2 are the additive and dominance variances, respectively, which sum to the marginal variance for locus *B;* i.e., the variance between the means for the columns in table 1. The partitioning of the variance to this point is the same as that of FISHER (1918) and WRIGHT (1935).

The last four components (σ_5^2 through σ_8^2) account for the remaining or epistatic portion of the variance of Y. The naming of the epistatic components corresponds to the relationships among the orthogonal scales:

$W_5 = W_1 \times W_3$ (additive × additive)
$W_6 = W_1 \times W_4$ (additive × dominance)
$W_7 = W_2 \times W_3$ (dominance × additive)
$W_8 = W_2 \times W_4$ (dominance × dominance).

The epistatic variance for the case of two loci, therefore, consists of four parts: σ_5^2 is the additive in *A* by additive in *B*, σ_6^2 is the additive in *A* by dominance in *B*, σ_7^2 is the dominance in *A* by additive in *B* and σ_8^2 is the dominance in *A* by dominance in *B*. FISHER (1918) and other subsequent workers, in expressing the epistatic variance for two loci, obtained one epistatic component which is actually the sum of the four components indicated above.

The epistatic components arise because the effects of genes at one locus depend on what genes are present at the other locus. For example, the dominance by dominance component is

$$\sigma^2_{\epsilon} = \left(\frac{f_{2.} f_{1.} f_{0.}}{4uv - f_{1.}}\right)\left(\frac{f_{.2} f_{.1} f_{.0}}{4xy - f_{.1}}\right)(e_{22} - e_{21} - e_{12} + e_{11})^2,$$

where the e's designate the following comparisons among the hereditary values:

$$\begin{aligned} e_{22} &= Y_{22} - Y_{21} - Y_{12} + Y_{11} \\ e_{21} &= Y_{21} - Y_{20} - Y_{11} + Y_{10} \\ e_{12} &= Y_{12} - Y_{11} - Y_{02} + Y_{01} \\ e_{11} &= Y_{11} - Y_{10} - Y_{01} + Y_{00}. \end{aligned}$$

All four epistatic components of variance are functions of these four comparisons, and unless each of the comparisons is zero there will be epistatic variance. The comparison e_{22}, for example, corresponds to $(AABB - AABb) - (AaBB - AaBb)$, and will be zero only if the gene effect at the B locus, $BB - Bb$, is the same for each of the phases, AA and Aa, at the A locus. The other comparisons bear similar interpretations and each represents the failure of the effect of a gene replacement at one locus to remain the same when a gene is replaced at the other locus. The epistatic components of variance will be illustrated in more detail later; the essential feature here being that they all arise from non-allelic gene interactions.

The extension to 3 and more loci is apparent. For the case of 3 loci there are 3 additive components, 3 dominance components and 20 epistatic components. The 20 epistatic components are 3 a × a, 6 a × d, 3 d × d, 1 a × a × a, 3 a × a × d, 3 a × d × d and 1 d × d × d (a = additive, d = dominance). For the case of n loci there are $3^n - 1$ components consisting of n components of the additive type, n components of the dominance type and $3^n - 2n - 1$ epistatic components. Of the $3^n - 2n - 1$ epistatic components there are $2n(n-1)$ two-factor components of which one-fourth are of the type a × a, one-half are of the type a × d and one-fourth are of the type d × d; $\frac{4}{3}n(n-1)(n-2)$ three-factor components of which one-eighth are of the type a × a × a, three-eighths are of the type a × a × d, three-eighths are of the type a × d × d and one-eighth are of the type d × d × d; etc. For many purposes the components of the same type may be combined. The types are designated separately because they present different properties in the correlations among relatives which will be discussed later.

Although the method presented here does not include multiple alleles, FISHER (1918) did partition the marginal variance for a locus with any number of alleles in a random mating population into an additive part and a dominance part. Correlations among the additive deviations and dominance deviations of relatives in a randomly mating population were the same as those for two alleles at a locus. It may be possible to partition the epistatic variance for multiple alleles by multiple regression and correlation techniques into components which will bear definitions similar to those where only two alleles are considered. Should the correlations among these epistatic deviations of relatives

be the same as those when only two alleles are considered, the development herein is general for any number of alleles. At present, this must remain as a conjecture.

Explicit expressions (in terms of Y's, gene frequencies and WRIGHT's inbreeding coefficient, F) are given in table 3 for the eight components of variance for the case of two loci and for any amount of inbreeding. In an inbred population the marginal frequencies for the *A* locus are $f_{2.} = u^2 + Fuv$, $f_{1.} = 2uv(1 - F)$ and $f_{0.} = v^2 + Fuv$, and those for the *B* locus are $f_{.2} = x^2 + Fxy$, $f_{.1} = 2xy(1 - F)$ and $f_{.0} = y^2 + Fxy$. When the marginal frequencies are uncorrelated, which is the case under consideration throughout this study, the joint frequencies are found by the simple relationship, $f_{ij} = f_{i.}f_{.j}$.

The partitions for random mating can be found from those in table 3 by letting F = 0. It should be recognized that when there is interaction among loci the marginal means, $Y_{i.}$ and $Y_{.j}$, vary with the degree of inbreeding, F. Thus one cannot compare the same marginal partitions for different degrees of inbreeding without writing out the marginal means in more detail. The Y_{ij}'s are also average values, averaged over the various gene combinations at other loci and over the various environmental sources of variation which determine the population of phenotypes. If interactions of 3 or more factors involve these loci, the Y_{ij}'s will also change with inbreeding. Thus the partitions of variance are descriptions of the population under consideration at the moment and will change with inbreeding.

How the inbreeding affects the partitions, or at least the ultimate end of the partitions as inbreeding approaches one, can be seen in table 3. The dominance variance disappears when F = 1. The disappearance is not linear with F, however. With some frequencies the dominance variance will actually increase for a period of time with inbreeding (ROBERTSON 1952). The last three of the epistatic partitions in table 3 also disappear as inbreeding becomes complete. Note that these three partitions involve dominance in their nomenclature. Their disappearance is not linear with F and is too complicated to allow any lucid generalizations. In fact, all partitions involving dominance in their nomenclature, and for any number of loci, will disappear as inbreeding becomes complete. If all partitions involving dominance in their nomenclature are zero, the effect of inbreeding on the additive partitions is to increase the one-factor additive partitions by $1 + F$, the two-factor additive partitions by $(1 + F)^2$, the three-factor additive partitions by $(1 + F)^3$, and so on. However, when partitions involving dominance in their nomenclature are not zero, the influence of inbreeding on the additive type of partitions cannot be foretold without specifying the hereditary values and gene frequencies.

CORRELATIONS BETWEEN RELATIVES

The covariances (or correlations) between the phenotypes of relatives can be analyzed in terms of the covariances between their respective components: that is, between the hereditary, environmental, and interaction values of one relative and those of the other relative. Only the covariance between hereditary

TABLE 3

Partitions of the hereditary variance and the variances of the orthogonal scales for two loci and any degree of inbreeding (F).

Orthogonal scale	Partitions of the variance	σ_W^2
W_1 a	$\frac{2uv}{1+F}[(u+Fv)(Y_{2\cdot}-Y_{1\cdot})+(v+Fu)(Y_{1\cdot}-Y_{0\cdot})]^2$	$2uv(1+F)$
W_2 d	$\frac{uv(u+Fv)(v+Fu)(1-F)}{1+F}(Y_{2\cdot}-2Y_{1\cdot}+Y_{0\cdot})^2$	$\frac{1+F}{uv(1-F)(u+vF)(v+uF)}$
W_3 a	$\frac{2xy}{1+F}[(x+Fy)(Y_{\cdot 2}-Y_{\cdot 1})+(y+Fx)(Y_{\cdot 1}-Y_{\cdot 0})]^2$	$2xy(1+F)$
W_4 d	$\frac{xy(x+Fy)(y+Fx)(1-F)}{1+F}(Y_{\cdot 2}-2Y_{\cdot 1}+Y_{\cdot 0})^2$	$\frac{1+F}{xy(1-F)(x+Fy)(y+Fx)}$
W_5 a × a	$\frac{4uvxy}{(1+F)^2}[(u+Fv)(x+Fy)e^*_{22}+(u+Fv)(y+Fx)e_{21}+(v+Fu)(x+Fy)e_{12}+(v+Fu)(y+Fx)e_{11}]^2$	$4uvxy(1+F)^2$
W_6 a × d	$\frac{2uvxy(1-F)(x+Fy)(y+Fx)}{(1+F)^2}[(u+Fv)(e_{22}-e_{21})+(v+Fu)(e_{12}-e_{11})]^2$	$\frac{2uv(1+F)^2}{xy(1-F)(x+Fy)(y+Fx)}$
W_7 d × a	$\frac{2xyuv(1-F)(u+Fv)(v+Fu)}{(1+F)^2}[(x+Fy)(e_{22}-e_{12})+(y+Fx)(e_{21}-e_{11})]^2$	$\frac{2xy(1+F)^2}{uv(1-F)(u+Fv)(v+Fu)}$
W_8 d × d	$\frac{uvxy(1-F)^2}{(1+F)^2}(x+Fy)(y+Fx)(u+Fv)(v+Fu)(e_{22}-e_{21}-e_{12}+e_{11})^2$	$\frac{(1+F)^2}{uvxy(1-F)^2(x+Fy)(y+Fx)(u+Fv)(v+Fu)}$

*The e's indicate the following comparisons among the hereditary values:

$e_{22} = Y_{22} - Y_{21} - Y_{12} + Y_{11}$ $\quad$ $e_{21} = Y_{21} - Y_{20} - Y_{11} + Y_{10}$

$e_{12} = Y_{12} - Y_{11} - Y_{02} + Y_{01}$ $\quad$ $e_{11} = Y_{11} - Y_{10} - Y_{01} + Y_{00}$

values will be considered here. The other covariances can sometimes be assumed to be zero, but this will depend on the organism and the circumstances. In human and livestock populations these correlations are often a troublesome feature. For example, in multiparous species, the environments of littermates are correlated. It is always necessary to be wary about assuming that the only covariances between relatives are those between their heredities.

The hereditary deviations of individuals are products of the regression coefficients, β_{YW}'s, and the appropriate scale values, W's. For example, the additive genetic deviation of an individual for the *A* locus is $\beta_{YW_1}W_1$, where W_1 is 2v, v-u or -2u corresponding to whether the individual is *AA*, *Aa* or *aa*, respectively. WRIGHT's (1935, 1950) additive estimates, G's in his notation, are the additive genetic deviations plus the mean, $\overline{Y}$. His dominance deviations, D = H – G in his notation, are the same as the present dominance deviations; for example, $\beta_{YW_2}W_2$'s for the *A* locus. An individual's hereditary value is a sum of the mean and its hereditary deviations,

$$Y = \overline{Y} + \sum_t \beta_{YW_t} W_t.$$

The correlations between the additive genetic and between the dominance deviations of relatives in a randomly mating population were given by FISHER (1918). He also gave the covariance between epistatic deviations of relatives for the case of two loci. WRIGHT (1922), with his coefficient of relationship, extended the correlations between additive genetic deviations of relatives to include inbreeding. WRIGHT's coefficient of relationship is the same as the absolute value of the correlation between additive genetic deviations of relatives as described in this paper. COCKERHAM (1952) gave the correlations between epistatic deviations of relatives in a randomly mating population.

The orthogonal scales may again be invoked to obtain these relationships for a randomly mating population or an inbreeding population where gene frequency is not changing, and to obtain the correlations among relatives for the epistatic deviations. Although one cannot find a joint frequency distribution of relatives which is general for all systems of inbreeding they can be computed for any particular system of consanguine mating. Again, the case of two loci with two genes each will be used for illustrative purposes; however, the extension to any number of loci will be apparent.

Let Y and Y′ be the hereditary values for two relatives (parent and offspring, full-sibs, etc.) and let W_t and $W_{t'}$ be the orthogonal scales of their respective generations. The scale values (terms of the scale) vary with the genotypes of course, but the entire scale may vary with the generation (e.g., when there is inbreeding). In a randomly mating population where gene frequencies do not change, relatives with the same genotype have the same hereditary deviations and the same scale values. The different notation (prime) simply designates whether the hereditary deviation or scale value is used for one relative or the other relative. In any system of inbreeding relatives with

the same genotype will have the same deviations and the same scale values provided they are in the same generation but can have different deviations and different scale values if they are in different generations, even though gene frequency does not change.

The covariance between Y and Y′ may be expressed as

$$\text{Cov } YY' = \sum_{t,t'} \text{Cov}(\beta_{Y W_t} W_t)(\beta_{Y' W'_{t'}} W'_{t'}), \quad (t, t' = 1 \ldots 8)$$

which is equivalent to

$$\sum_{t,t'} \rho(\beta_{Y W_t} W_t)(\beta_{Y' W'_{t'}} W'_{t'}) \sigma_t \sigma'_{t'}$$

because $\sigma_t^2 = \beta_{Y W_t}^2 \sigma_{W_t}^2$. Now, $\rho(\beta_{Y W_t} W_t)(\beta_{Y' W'_{t'}} W'_{t'}) = \pm \rho_{W_t W'_{t'}}$

because the regression coefficients are constants and do not affect the absolute value of the correlation coefficients. The sign (+ or −) will be the same as the sign of the product of the two regression coefficients, and will be considered in more detail later. It is necessary then to determine the correlation between the eight orthogonal scales of one relative and the eight orthogonal scales of the other relative. This is done by constructing a nine by nine joint distribution table for the two relatives. Although the results are simple, the development is involved and is given in an Appendix. If the genotypic frequencies for all relatives are proportional to the marginal frequencies of the loci, if the genes recombine independently, and if gene frequencies do not change, the correlations between the epistatic scales of two relatives are products of the one-factor correlations as given in table 4.

TABLE 4

Correlations between the eight scales of one relative and the eight scales of another relative.

	W'_1	W'_2	W'_3	W'_4	W'_5	W'_6	W'_7	W'_8
W_1	$\rho_{W_1 W'_1}$	$\rho_{W_1 W'_2}$	0	0	0	0	0	0
W_2	$\rho_{W_2 W'_1}$	$\rho_{W_2 W'_2}$	0	0	0	0	0	0
W_3	0	0	$\rho_{W_3 W'_3}$	$\rho_{W_3 W'_4}$	0	0	0	0
W_4	0	0	$\rho_{W_4 W'_3}$	$\rho_{W_4 W'_4}$	0	0	0	0
W_5	0	0	0	0	$\rho_{W_1 W'_1} \rho_{W_3 W'_3}$	$\rho_{W_1 W'_1} \rho_{W_3 W'_4}$	$\rho_{W_1 W'_2} \rho_{W_3 W'_3}$	$\rho_{W_1 W'_2} \rho_{W_3 W'_4}$
W_6	0	0	0	0	$\rho_{W_1 W'_1} \rho_{W_4 W'_3}$	$\rho_{W_1 W'_1} \rho_{W_4 W'_4}$	$\rho_{W_1 W'_2} \rho_{W_4 W'_3}$	$\rho_{W_1 W'_2} \rho_{W_4 W'_4}$
W_7	0	0	0	0	$\rho_{W_2 W'_1} \rho_{W_3 W'_3}$	$\rho_{W_2 W'_1} \rho_{W_3 W'_4}$	$\rho_{W_2 W'_2} \rho_{W_3 W'_3}$	$\rho_{W_2 W'_2} \rho_{W_3 W'_4}$
W_8	0	0	0	0	$\rho_{W_2 W'_1} \rho_{W_4 W'_3}$	$\rho_{W_2 W'_1} \rho_{W_4 W'_4}$	$\rho_{W_2 W'_2} \rho_{W_4 W'_3}$	$\rho_{W_2 W'_2} \rho_{W_4 W'_4}$

Two simple generalizations are apparent from table 4. (1) One-factor deviations (additive or dominance) of one relative are correlated, if at all, only with one-factor deviations of the other relative, and also the deviations must pertain to the same locus. This is actually a particular case of the broader generalization that n-factor deviations of one relative are correlated, if at all, only with n-factor deviations of the other relative, and the deviations for both

relatives must involve the same loci. (2) The correlations between the epistatic or multi-factor deviations are functions of the correlations between one-factor deviations.

Relatives in a randomly mating population

If the one-factor additive deviations of either relative are uncorrelated with the one-factor dominance deviations of the other relative, i.e., $\rho_{W_1W_2'} = \rho_{W_2W_1'} = 0$ for all loci, then all the correlations in table 4 are zero, except for the diagonal ones. This means that a deviation in one relative is correlated, if at all, only with the same deviation in the other relative. This condition is fulfilled in a randomly breeding population. Since the one-factor additive correlations are the same and the one-factor dominance correlations are the same for all loci, a simple rule can be used for computing the various correlations. Let p be the correlation between one-factor additive deviations of the relatives, and let q be the correlation between one-factor dominance deviations of the relatives. The correlation between any type of deviation of the relatives is

$$(p)^A(q)^D,$$

where A is the number of factors (loci) entering into the deviation with additive nomenclature and D is the number of factors entering into the deviation with dominance nomenclature. The deviation is an (A + D)-factor deviation. When the two relatives are parent and offspring, p = ½ and q = 0. No deviations involving dominance in their nomenclature are correlated between parent and offspring. The correlations between the indicated types of deviations are:

$$\begin{array}{ccc} a & a \times a & a \times a \times a \\ \left(\frac{1}{2}\right)^1 (0)^0 = \frac{1}{2} & \left(\frac{1}{2}\right)^2 (0)^0 = \frac{1}{4} & \left(\frac{1}{2}\right)^3 (0)^0 = \frac{1}{8} \end{array}$$

Dominance is illustrated by considering the relationships between full sibs; i.e. $p = \frac{1}{2}$ and $q = \frac{1}{4}$. The correlations between the epistatic deviations are

$$\begin{array}{ccc} a \times a & a \times d & d \times d \\ \left(\frac{1}{2}\right)^2 \left(\frac{1}{4}\right)^0 = \frac{1}{4} & \left(\frac{1}{2}\right)^1 \left(\frac{1}{4}\right)^1 = \frac{1}{8} & \left(\frac{1}{2}\right)^0 \left(\frac{1}{4}\right)^2 = \frac{1}{16}, \text{ and so on.} \end{array}$$

The correlations between the various deviations of relatives in a randomly mating population are all positive, since each one involves the same deviation and the two regression coefficients, β_{YW} and $\beta_{Y'W'}$, are identical in each case.

Since the partitions of the variance are the same for each relative in a randomly mating population the covariance between the hereditary values of two relatives can be given in terms of partitions of the hereditary variance. Also since the correlations among hereditary deviations are the same for all

those of the same type it is convenient to designate the sum of the components of variance of the same type by a single term. Let σ_{AD}^2 be the sum of all the components in whose nomenclature additive appears A times and dominance appears D times. For example, $\sigma_{10}^2 (A = 1, D = 0)$ is the additive genetic variance which is the sum of all the one-factor additive components (one from each locus), σ_{01}^2 is the dominance variance, σ_{11}^2 is the a × d epistatic variance, and so on. Symbolically, the total hereditary variance is

$$\sigma_Y^2 = \sum_{\substack{A,D=0 \\ 1 \leq A+D \leq n}}^{n} \sigma_{AD}^2,$$

where n is the number of loci. The summation is over both A and D where each varies from 0 to n but subject to the limitations that for any term A and D cannot both be zero nor can their sum be greater than n. The covariance between relatives can be given in terms of these partitions,

$$\text{Cov } YY' = \sum_{\substack{A,D=0 \\ 1 \leq A+D \leq n}}^{n} p^A q^D \sigma_{AD}^2.$$

When the two relatives are parent and offspring the covariance reduces to

$$\sum^{n} \left(\frac{1}{2}\right)^A \sigma_{AO}^2,$$

which is one-half the additive genetic variance, plus one-fourth the a × a variance plus one-eighth the a × a × a variance, and so on.

If only the heredities of relatives are correlated, the covariance between their phenotypes is the same as the covariance between their heredities. In this case the phenotypic correlation or the phenotypic regression of one relative on the other is the ratio of the covariance between the heredities of the relatives to the total phenotypic variance.

Relatives in a self-fertilizing population

Although the correlations among epistatic deviations of relatives in an inbred population are functions of the correlations for one-factor deviations, several new considerations are involved. The absolute values of the correlations between one-factor additive deviations are the same as WRIGHT'S (1922) coefficient of relationship (the correlations can be negative when the relatives are in different generations depending on dominance and epistatic effects of the genes). This means that the absolute values of the correlations between the epistatic deviations of relatives involving only additive in their nomenclature can be found in the same manner as when mating is random. The correlations between

one-factor dominance deviations of relatives must be computed for each system of inbreeding and involve considerable labor. Also, one-factor additive deviations of one relative are correlated with one-factor dominance deviations of the other relative. This means that many more of the epistatic deviations of one relative are correlated with those of the other relative than in a randomly breeding population.

Self-fertilization was chosen as the system of inbreeding to illustrate the correlations among relatives in an inbred population for three main reasons. First, it is the most extreme form of inbreeding and relationships in a milder system of inbreeding should fall between these for selfing and those indicated earlier for random mating; second, considerable work is being done in plant breeding with normally selfed organisms or by selfing organisms; and third, it is by far the easiest system to work with computationally. The correlations between hereditary deviations of parent and of offspring will be considered at first.

The correlation between one-factor additive scales of the parent and one-factor additive scales of the offspring is

$$\frac{1 + F' + 2F}{2\sqrt{1 + F}\ \sqrt{1 + F'}},$$ (LUSH 1948)

where F' and F are the inbreeding coefficients of the parent and the offspring, respectively. When selfing

$$F = \frac{1 + F'}{2},$$

so that the correlation becomes

$$\sqrt{\frac{2F}{1 + F}}.$$

The correlation between the one-factor dominance scales of parent and of offspring in a self-fertilizing population is

$$\frac{1}{2}\sqrt{\frac{(1 + F)(u - v + 2Fv)(v - u + 2Fu)}{F(u + Fv)(v + Fu)}},$$

which involves gene frequency, u and v at the A locus in this example. Also in a self-fertilizing population, the one-factor additive scales of the parent may be correlated with the one-factor dominance scales of the offspring. This correlation is

$$\frac{(u - v)(1 - F)}{2}\sqrt{\frac{(1 - F)}{F(1 + F)(u + Fv)(v + Fu)}},$$

which is zero when gene frequency is one-half. The dominance scales of the parent and the additive scales of the offspring are not correlated.

The correlations among the epistatic scales can be found from the one-factor correlations by substitution in table 4. The simple rule used for computing the correlation between any type of deviation in a randomly mating population cannot be used here because (1) the one-factor dominance correlations involve gene frequencies and can differ from locus to locus, (2) different types of epistatic deviations may be correlated, and (3) in terms of hereditary deviations the correlations may be negative.

The signs (+ or −) of the correlations vary with the dominance and epistatic effects of the genes. For example, even in the absence of epistasis, the correlation between the additive deviations of non-inbred parents and first generation selfed offspring is negative for an over-dominant locus when the frequency of the favorable gene is slightly higher than the frequency that would make the mean of the parents a maximum. For any given situation the sign can be found and is the same as the sign of the product of the two regression coefficients, β_{YW} and $\beta_{Y'W'}$, corresponding to the deviations under question. When the relatives are in the same generation the correlation between the same type of deviation will always be positive because the two regression coefficients are identical, but even here the signs of the correlations between different types of deviations have to be determined and also all of the correlations involve gene frequency. The knowledge required to determine the correlations, gene frequencies and regression coefficients, precludes any use of this type of biometrical analysis, with one exception, and this is when all gene frequencies are one-half. In this case a deviation of one relative is correlated, if at all, only with the same deviation of the other relative, as is true for relatives in a randomly mating population.

The frequencies of segregating genes can be reasonably assumed to be one-half in subsequent generations of a cross between two homozygous lines. However, the applicability of the following results is limited somewhat because of the assumption, necessary in this analysis, that the genotypic frequencies are proportional to the marginal frequencies of the loci. Even with random mating following the cross it is several generations before the genotypic frequencies can be reasonably assumed to be proportional to the marginal frequencies of those loci which are closely linked. When generations subsequent to the cross are selfed or inbred rapidly the genotypic frequencies cannot ever be reasonably assumed to be proportional to the marginal frequencies of the linked loci because the parental types are fixed much more rapidly than the recombinants. In this case the partitions of the variance and the following correlations among relatives apply strictly only to those combinations of loci whose genes recombine independently.

Where each generation after the first generation of a cross between two homozygous parental lines is obtained by self-fertilizing the previous generation, the coefficient of inbreeding in the gth generation is

$$\frac{2^{g-2} - 1}{2^{g-2}}$$

The correlation between the parent in the $(g-1)$th generation and the offspring in the gth generation is then:

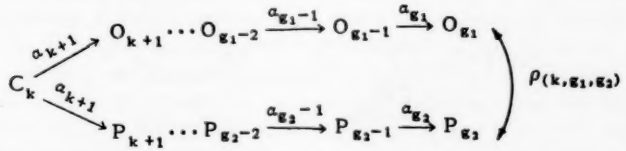
$$\sqrt{\frac{2(2^{g-2}-1)}{2^{g-1}-1}}$$

for the additive scales, and

$$\sqrt{\frac{2^{g-2}-1}{2^{g-1}-1}}$$

for the dominance scales. These correlations have meaning only when g is greater than two.

By using the correlations between parent and offspring a more general scheme can be developed for indicating the correlations between any two relatives. Let $\rho_{a(k,g_1,g_2)}$ be the correlation between the additive scales of one relative, O_{g_1}, in the g_1th generation and of another relative, P_{g_2}, in the g_2th generation both of whom descended from the last common parent, C_k, in the kth generation. Let $\rho_{d(k,g_1,g_2)}$ be the correlation between the dominance scales of the same two relatives. The following path coefficient diagram shows the relationships between these relatives.



P's are used to indicate the parents of P_{g_2} and O's are used to indicate the parents of O_{g_1} in their descent from the last common parent in the kth generation. The single-headed arrows are path coefficients (WRIGHT 1934), and the double headed arrow indicates a correlation coefficient. The only connection between the genotypes of the two relatives is through the chain of parents via the common parent. Since the additive scales of one individual are correlated only with the additive scales (and not the dominance scales) of the preceding parent (or succeeding offspring), the path coefficient, α, for the additive scales is actually the correlation coefficient between the additive scales of the parent and of the offspring. The correlation between the additive scales of O_{g_1} and P_{g_2} is computed by multiplying together all the path coefficients:

$$\rho_{a(k,g_1,g_2)} = \alpha_{g_1}\,\alpha_{g_1-1} \cdots \alpha^2_{k+1} \cdots \alpha_{g_2-1}\,\alpha_{g_2}.$$

Substituting

$$\alpha_g = \sqrt{\frac{2(2^{g-2}-1)}{2^{g-1}-1}},$$

the correlation between the additive scales of the two relatives is

$$\rho_{a(k,g_1,g_2)} = \frac{2^{(g_1+g_2-2k)/2}\,(2^{k-1}-1)}{\sqrt{(2^{g_1-1}-1)\,(2^{g_2-1}-1)}}.$$

The correlation between the dominance scales of O_{g_1} and P_{g_2} are found in the same manner, except of course, the path coefficients are correlations between the dominance scales. Substituting

$$\alpha_g = \sqrt{\frac{2^{g-2}-1}{2^{g-1}-1}},$$

the correlation between the dominance scales of the two relatives is

$$\rho_{d(k,g_1,g_2)} = \frac{2^{k-1}-1}{\sqrt{(2^{g_1-1}-1)\,(2^{g_2-1}-1)}}.$$

The correlation between the various epistatic scales can be found by the familiar formula:

$$\left[\rho_{a(k,g_1,g_2)}\right]^A \left[\rho_{d(k,g_1g_2)}\right]^D$$

When the relatives are in the same generation the covariance between their hereditary values can be given in terms of partitions of the hereditary variance,

$$\text{Cov } YY' = \sum_{\substack{A,D=0 \\ 1\le A+D\le n}}^{n} \rho^A_{a(k,g,g)}\,\rho^D_{d(k,g,g)}\,\sigma^2_{AD},$$

as was done for the randomly mating population. Remember that these partitions of variance are for the inbred populations and change with the degree of inbreeding. As an example of the procedure, consider a population of F_4 individuals which are classified according to their F_3 parents and F_2 grandparents. The total hereditary variation of these F_4 individuals is distributed in the following analysis of variance as

Source	*Variance*
Between F_2 progeny means	$\sum_{A,D} \rho^A_{a(2,4,4)}\,\rho^D_{d(2,4,4)}\,\sigma^2_{AD}$
Between F_3 progeny means within F_2 parents	$\sum_{A,D}\left[\rho^A_{a(3,4,4)}\,\rho^D_{d(3,4,4)} - \rho^A_{a(2,4,4)}\,\rho^D_{d(2,4,4)}\right]\sigma^2_{AD}$
Between F_4 individuals with the same F_3 parent	$\sum_{A,D}\left[1 - \rho^A_{a(3,4,4)}\,\rho^D_{d(3,4,4)}\right]\sigma^2_{AD}$,
where	$\sum_{A,D} \rho^A_{a(2,4,4)}\,\rho^D_{d(2,4,4)}\,\sigma^2_{AD}$

is the covariance between the hereditary values of F_4 individuals which descended from the same F_2 grandparents but different F_3 parents, and

$$\sum_{A,D} \rho^{A}_{a(3,4,4)} \rho^{D}_{d(3,4,4)} \sigma^{2}_{AD}$$

is the covariance between the hereditary values of F_4 individuals which descended from the same F_3 parent. The one-factor correlations are $\rho_{a(2,4,4)} = 4/7$, $\rho_{a(3,4,4)} = 6/7$, $\rho_{d(2,4,4)} = 1/7$ and $\rho_{d(3,4,4)} = 3/7$.

Relatives from randomly mated inbred parents

The final situation to be considered is one in which several inbred lines are made from a randomly mating population. The inbreeding program is interrupted at any desired stage of inbreeding to cross the inbred individuals at random. Although the parents are inbred, the offspring will not be. Barring selection and linkage, the offspring will reconstitute the original randomly breeding population from which the inbred individuals were obtained. Whether one can actually obtain unselected inbred lines or individuals is questionable. If the genotypic frequencies are proportional to the marginal frequencies of the loci and if gene frequencies do not change (no selection), the gametic arrays from any generation of inbreeding will be the same as those in the randomly mating population. Thus the offspring from the randomly mated inbred parents will be a reconstitution of the original population. However, the genotypic frequencies among the inbred parents, at least when the parents are the result of self-fertilization, will not be proportional to the marginal frequencies of those loci which have recombination values less than one-half. This will affect the joint distribution of full sib offspring and of half sib offspring. In a few examples considered, the effect on the joint distribution is small. Nevertheless, if the inbreeding is between zero and one ($0 < F < 1$), the results are accurate only for those combinations of loci which have recombination frequencies of one-half. The frequencies of genotypes among parents inbred to homozygosity ($F = 1$) are proportional to the marginal frequencies of the loci, regardless of linkage relationships, if the genotypic frequencies in the original population were proportional to the marginal frequencies of the loci and if there has been no selection or change in gene frequencies.

The covariance between offspring with both parents in common, full sibs, and the covariance between offspring with one parent in common, half sibs, will be considered. The joint distribution of full sibs from randomly mated inbred parents for the *A* locus is given in table 5. From this table the following correlations are found: $\rho_{W_1W_{1'}} = (1+F)/2$, $\rho_{W_1W_{2'}} = \rho_{W_2W_{1'}} = 0$, $\rho_{W_2W_{2'}} = [(1+F)/2]^2$, i.e., the correlation between the additive deviations of full sibs is $(1+F)/2$, the additive deviations of one full sib are not correlated with the dominance deviations of the other full sib, and the correlation between the dominance deviations is the square of the correlation between the additive deviations.

In an analogous manner it is found that of the one-factor deviations only the additive deviations of half sibs are correlated, and this correlation is $(1+F)/4$.

TABLE 5

Joint frequency of full sibs from randomly mated inbred parents (A locus).

	Full sib′			Genetic type	f	W_1	W_2
Full sib	$u^2[u + Fv + v^2(1 - F)^2/4]$	$u^2v(1 - F)(1 + u + Fv)/2$	$u^2v^2(1 - F)^2/4$	*AA*	u^2	2v	$1/u^2$
	$u^2v(1 - F)(1 + u + Fv)/2$	$uv[1 + F + uv(1 - F)^2]$	$uv^2(1 - F)(1 + v + Fu)/2$	*Aa*	2uv	v-u	-1/uv
	$u^2v^2(1 - F)^2/4$	$uv^2(1 - F)(1 + v + Fu)/2$	$v^2[v + Fu + u^2(1 - F)^2/4]$	*aa*	v^2	-2u	$1/v^2$
Genetic Type	*AA*	*Aa*	*aa*				
f′	u^2	2uv	v^2				
W_1'	2v	v-u	-2u				
W_2'	$1/_u2$	-1/uv	$1/_v2$				

The correlations between the epistatic deviations are found in the manner given previously: $(p)^A(q)^D$. The correlations are all positive.

When the parents are inbred to homozygosity, F = 1, the correlations between hereditary deviations of half sibs are the same as those between parent and offspring in the original population. This is a reasonable result when one considers that the relationship, in both cases, is between two individuals in a randomly mating population with a gamete in common.

Again, the covariance between the hereditary values of these relatives, which is the same as the covariance between their phenotypes if only their hereditary values are correlated, can be expressed in the familiar form,

$$\text{Cov } YY' = \sum_{\substack{A,D=0 \\ 1 \leq A+D \leq n}}^{n} p^A q^D \sigma^2_{AD}.$$

The partitions of variance are the same as those in the original randomly mating population.

SUMMARY

The hereditary variance of diploid populations, whose genotypic frequencies are proportional to the marginal frequencies of the loci, was partitioned into $3^n - 1$ partitions analogous to the linear and quadratic analysis of a 3^n factorial representation, where n is the number of loci (factors) each with three genetic phases or levels (no multiple alleles). These partitions are grouped into types of which there are $s + 1$ types of s-factor partitions. The one-factor types are (1) additive (linear) variance, or additive genetic variance, which is the sum of the n partitions, one for each locus, resulting from additive effects of genes and (2) dominance (quadratic) variance which is the sum of the n partitions resulting from dominance or allelic interactions of genes. The remaining two and more factor partitions result from non-allelic interactions of genes and constitute the epistatic variance. The three types of two-factor epistatic variances are (1) additive by additive variance which is the sum of the $n(n-1)/2$ partitions, one for each combination of two loci, resulting from joint additive by additive effects of non-allelic genes, (2) additive by dominance variance which is the sum of the $n(n-1)$ partitions resulting from joint additive by dominance effects of non-allelic genes and (3) dominance by dominance variance which is the sum of the $n(n-1)/2$ partitions resulting from joint dominance by dominance effects of non-allelic genes. The three (or more) factor types of epistatic variance bear similar interpretations.

Correlations between the hereditary deviations (a, d, a × a, a × d, and so on; a = additive, d = dominance) of relatives were considered for the following situations: (1) relatives in a randomly mating population, (2) relatives in a self-fertilizing population and (3) relatives which are offspring from randomly mated inbred parents. For situations 1 and 3, and for situation 2 when gene frequencies are one-half and the relatives are in the same generation, the corre-

lations between the epistatic deviations of the relatives are products of the one-factor additive and dominance correlations. For each of these situations only the same types of deviations involving the same locus or loci are correlated and the correlations are the same for deviations of the same type. Therefore, the correlations between the hereditary deviations of the relatives may be generally expressed as

$$(p)^A(q)^D,$$

where additive appears A times and dominance appears D times in the nomenclature of the deviation, and where p and q are the correlations between the additive and dominance deviations, respectively. Thus the covariance between hereditary values of the two relatives is

$$\sum_{\substack{A,D=0 \\ 1\leq A+D\leq n}}^{n} p^A q^D \sigma^2_{AD}$$

For example, where the two relatives are parent and offspring in a randomly mating population, $p = 1/2$ and $q = 0$. The covariance between the hereditary values of parent and offspring is $\sum_{A=1}^{n} (1/2)^A \sigma^2_{AO}$, which is 1/2 the additive genetic variance, plus 1/4 the a × a variance, plus 1/8 the a × a × a variance, and so on.

LITERATURE CITED

COCKERHAM, C. CLARK, 1952 Genetic covariation among characteristics of swine. Unpublished Ph.D. Thesis. Iowa State College Library. Ames, Iowa.

FISHER, R. A., 1918 The correlation between relatives on the supposition of Mendelian inheritance. Trans. Roy. Soc. Edinburgh **52**: 399–433.

LUSH, J. L., 1948 The genetics of populations. Ames, Iowa. Mimeo.

ROBERTSON, ALAN, 1952 The effect of inbreeding on the variation due to recessive genes. Genetics **37**: 189–207.

NELDER, J. A., 1953 Statistical models in biometrical genetics. Heredity **7**: 111–119.

SNEDECOR, G. W., 1946 Statistical methods, 4th edition. Iowa State College Press. Ames, Iowa.

WRIGHT, SEWALL, 1922 Coefficients of inbreeding and relationship. Am. Nat. **56**: 330–338.

1934 The method of path coefficients. Ann. Math. Stat. **5**: 161–215.

1935 The analysis of variance and the correlations between relatives with respect to deviations from an optimum. J. Genet. **30**: 243–256.

1950 The genetics of quantitative variability. Colloquium at the Institute of Animal Genetics of the University of Edinburgh, 1949–1950.

APPENDIX

GENERAL DERIVATION OF THE CORRELATIONS BETWEEN SCALES OF RELATIVES

Consider at first the joint distribution of two relatives for the A locus averaged over all other loci, table 6. The h's are the joint frequencies of the two relatives; for example,

TABLE 6

Joint frequency of two relatives (A locus).

	Relative′			Genetic type	f	W_1	W_2
Relative	h_{22}	h_{21}	h_{20}	*AA*	$f_{2.}$	2v	$1/f_{2.}$
	h_{12}	h_{11}	h_{10}	*Aa*	$f_{1.}$	v-u	$-2/f_{1.}$
	h_{02}	h_{01}	h_{00}	*aa*	$f_{0.}$	-2u	$1/f_{0.}$
Genetic type	*AA*	*Aa*	*aa*				
f′	$f'_{2.}$	$f'_{1.}$	$f'_{0.}$				
W'_1	2v	v-u	-2u				
W'_2	$1/f'_{2.}$	$-2/f'_{1.}$	$1/f'_{0.}$				

h_{22} is the frequency with which the *AA* relative appears with the *AA* relative′. The row and column frequencies add to the marginal frequencies of the relatives

$$f'_{i'.} = \sum_i h_{ii'}, \qquad f_{i.} = \sum_{i'} h_{ii'},$$

which are also the marginal frequencies for the *A* locus in each of the relative's generation. The correlation between a one-factor scale for the *A* locus of one relative and a one-factor scale for the *A* locus of the other relative is

$$\rho_{WW'} = \frac{\sum_{i,i'} h_{ii'} W_i W'_{i'}}{\sqrt{\sum_i f_{i.} W_i^2 \sum_{i'} f'_{i'.} (W'_{i'})^2}} = \frac{\text{Cov } W W'}{\sigma_W \sigma_{W'}}. \tag{1}$$

As an example let the primed relative be the offspring and the unprimed relative be the parent in a randomly mating population where the gene frequency is the same in the two generations. The joint frequencies are: $h_{22} = u^3$, $h_{21} = u^2v$, $h_{20} = 0$, $h_{12} = u^2v$, $h_{11} = uv$, $h_{10} = uv^2$, $h_{02} = 0$, $h_{01} = uv^2$, $h_{00} = v^3$. The correlations are from (1): $\rho_{W_2' W_1} = \frac{1}{2}$, $\rho_{W_1' W_2} = \rho_{W_2' W_1} = \rho_{W_2' W_2} = 0$.

The joint frequencies, $k_{jj'}$, of the two relatives for the B locus are shown similarly in table 7.

When the joint frequencies of relatives for two loci are proportional to the joint frequencies of the relatives for each locus, the joint frequencies for both loci (table 8) can be found by multiplying together the joint frequencies for each locus (tables 6 and 7). They are proportional in randomly mating populations and in inbreeding populations if gene frequencies are not changing, if genotypic frequencies in each generation are proportional to the marginal frequencies of loci and if loci are not linked. The frequency with which the *AABB* relative appears with the *AABB* relative′ is $h_{22} k_{22}$, and so on for the other genotypes. The frequency of the *AABB* relative is $f_{2.} f_{.2} = f_{22}$ and the frequency of the *AABB* relative′ is $f_{2.}' f_{.2}' = f_{22}'$. The joint frequencies for locus *A*

TABLE 7

Joint frequency of two relatives (B locus).

	Relative′			Genetic type	f	W_3	W_4
Relative	k_{22}	k_{21}	k_{20}	*BB*	$f_{.2}$	2y	$1/f_{.2}$
	k_{12}	k_{11}	k_{10}	*Bb*	$f_{.1}$	y-x	$-2/f_{.1}$
	k_{02}	k_{01}	k_{00}	*bb*	$f_{.0}$	-2x	$1/f_{.0}$
Genetic type	*BB*	*Bb*	*bb*				
f′	$f'_{.2}$	$f'_{.1}$	$f'_{.0}$				
W'_3	2y	y-x	-2x				
W'_4	$1/f'_{.2}$	$-2/f'_{.1}$	$1/f'_{.0}$				

are on the left side of the block of joint frequencies for locus *B* in table 8. To complete the table, the frequency for *A* must multiply each term in the block.

The covariance between a scale of one relative and that of the other relative is

$$\text{Cov } WW' = \sum_{i,i',j,j'} h_{ii'} k_{jj'} W_{ij} W'_{i'j'}. \tag{2}$$

These covariances simplify when we consider the relationships among the orthogonal scales. Only the first four scales are given for each relative in table 8 since these can be used to construct the remaining four which pertain to epistasis. Note in table 8 (and in table 3) that the individual terms of the scales have the following relationships:

$W_{ij} = W_{i.}$, or is constant for each i, for scales W_1 and W_2 relating to the *A* locus
$= W_{.j}$, or is constant for each j, for scales W_3 and W_4 relating to the *B* locus,

and remember that

$$W_{ij} = W_{i.} W_{.j} \text{ for } W_5, W_6, W_7 \text{ and } W_8 \text{ or the epistatic scales.}$$

(The subscripts i and j designate terms of a particular scale.) The same relationships hold among the W′s. Thus, $W_{i.} W_{.j}$ may be substituted for W_{ij} and $W_{i.'} W_{.j'}$ may be substituted for $W_{i'j'}$ in (2) for the epistatic scales. With these substitutions the covariance in (2) becomes

$$\text{Cov } WW' = \sum_{i,i'} h_{ii'} W_{i.} W'_{i'.} \sum_{j,j'} k_{jj'} W_{.j} W'_{.j'}. \tag{3}$$

But $\sum_{i,i'} h_{ii'} W_{i.} W'_{i'.}$ is the covariance pertaining to the one-factor scales for locus A in (1), and $\sum_{j,j'} k_{jj'} W_{.j} W'_{.j'}$ is the covariance pertaining to the one-factor scales for locus B, so that the covariances between the epistatic scales can be evaluated in terms of the covariances of the one-factor scales for each locus. For example,

$$\text{Cov } W_5 W'_5 = \sum_{i,i'} h_{ii'} W_{i1.} W'_{i'1.} \sum_{j,j'} k_{jj'} W_{3.j} W'_{3.j'}$$

$$= \text{Cov } W_1 W'_1 \text{ Cov } W_3 W'_3.$$

TABLE 8

Joint frequency of two relatives for two loci.

	Relative′										Genetic type	f	W_1	W_2	W_3	W_4
		k_{22}	k_{21}	k_{20}		k_{22}	k_{21}	k_{20}		k_{22}	k_{21}	k_{20}	*AABB*	f_{22}	2v	$1/f_{2\cdot}$	2y	$1/f_{\cdot 2}$
	h_{22}	k_{12}	k_{11}	k_{10}	h_{21}	k_{12}	k_{11}	k_{10}	h_{20}	k_{12}	k_{11}	k_{10}	*AABb*	f_{21}	2v	$1/f_{2\cdot}$	y-x	$-2/f_{\cdot 1}$
		k_{02}	k_{01}	k_{00}		k_{02}	k_{01}	k_{00}		k_{02}	k_{01}	k_{00}	*AAbb*	f_{20}	2v	$1/f_{2\cdot}$	-2x	$1/f_{\cdot 0}$
		k_{22}	k_{21}	k_{20}		k_{22}	k_{21}	k_{20}		k_{22}	k_{21}	k_{20}	*AaBB*	f_{12}	v-u	$-2/f_{1\cdot}$	2y	$1/f_{\cdot 2}$
Relative	h_{12}	k_{12}	k_{11}	k_{10}	h_{11}	k_{12}	k_{11}	k_{10}	h_{10}	k_{12}	k_{11}	k_{10}	*AaBb*	f_{11}	v-u	$-2/f_{1\cdot}$	y-x	$-2/f_{\cdot 1}$
		k_{02}	k_{01}	k_{00}		k_{02}	k_{01}	k_{00}		k_{02}	k_{01}	k_{10}	*Aabb*	f_{10}	v-u	$-2/f_{1\cdot}$	-2x	$1/f_{\cdot 0}$
		k_{22}	k_{21}	k_{20}		k_{22}	k_{21}	k_{20}		k_{22}	k_{21}	k_{20}	*aaBB*	f_{02}	-2u	$1/f_{0\cdot}$	2y	$1/f_{\cdot 2}$
	h_{02}	k_{12}	k_{11}	k_{10}	h_{01}	k_{12}	k_{11}	k_{10}	h_{00}	k_{12}	k_{11}	k_{10}	*aaBb*	f_{01}	-2u	$1/f_{0\cdot}$	y-x	$-2/f_{\cdot 1}$
		k_{02}	k_{01}	k_{00}		k_{02}	k_{01}	k_{00}		k_{02}	k_{01}	k_{00}	*aabb*	f_{00}	-2u	$1/f_{0\cdot}$	-2x	$1/f_{\cdot 0}$
Genetic type		*AA* *BB*	*AA* *Bb*	*AA* *bb*		*Aa* *BB*	*Aa* *Bb*	*Aa* *bb*		*aa* *BB*	*aa* *Bb*	*aa* *bb*						
f'		f'_{22}	f'_{21}	f'_{20}		f'_{12}	f'_{11}	f'_{10}		f'_{02}	f'_{01}	f'_{00}						
W'_1		2v	2v	2v		v-u	v-u	v-u		-2u	-2u	-2u						
W'_2		$1/f'_{2\cdot}$	$1/f'_{2\cdot}$	$1/f'_{2\cdot}$		$-2/f'_{1\cdot}$	$-2/f'_{1\cdot}$	$-2/f'_{1\cdot}$		$1/f'_{0\cdot}$	$1/f'_{0\cdot}$	$1/f'_{0\cdot}$						
W'_3		2y	y-x	-2x		2y	y-x	-2x		2y	y-x	-2x						
W'_4		$1/f'_{\cdot 2}$	$-1/f'_{\cdot 1}$	$1/f'_{\cdot 0}$		$1/f'_{\cdot 2}$	$-2/f'_{\cdot 1}$	$1/f'_{\cdot 0}$		$1/f'_{\cdot 2}$	$-2/f'_{\cdot 1}$	$1/f'_{\cdot 0}$						

The covariance between the additive by additive epistatic scales of one relative and the additive by additive epistatic scales of the other relative is the product of the covariance between the additive scales of the two relatives for one locus and of the covariance between the additive scales of the two relatives for the other locus. The same relationship exists between the correlations as between covariances,

$$\rho_{w_5\ w_{5'}} = \rho_{w_1\ w_{1'}} \rho_{w_3\ w_{3'}},$$

because $\sigma_{w_5} = \sigma_{w_1}\sigma_{w_3}$ and $\sigma_{w_{5'}} = \sigma_{w_{1'}}\sigma_{w_{3'}}$. The correlations between the eight scales of one relative and the eight scales of the other relative are given in table 4.

A MODEL FOR THE STUDY OF QUANTITATIVE INHERITANCE[1]*

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THE actions of genes controlling quantitative characters may be described by the use of gene models. FISHER (1918) constructed a gene model which included dominance at a single locus. He stated that there may be a deviation from the simple additive effects between loci, similar to dominance at one locus, if more than one locus affected a given character. He called the deviation epistacy and introduced the term dual epistacy to indicate the interaction of all possible pairs of loci affecting the given character. Later, FISHER, IMMER and TEDIN (1932) used this gene model to describe the actions of any number of genes on a given character assuming there was no epistacy.

GRIFFING (1950) introduced an epistatic parameter into a model, but the model is not easily extended to an arbitrary number of loci. The present paper contains a model based on the factorial model used in the design of experiments and some applications of this model to some data.

THE FACTORIAL GENE MODEL

The factorial model may be exemplified by the case of a dihybrid. The relative frequencies of the nine genotypes obtained from selfing the complete heterozygote may be represented in the following manner:

			locus *a*		
		aa	*Aa*	*AA*
		a_0	a_1	a_2
locus *b*	*bb*	b_0	1	2	1
	Bb	b_1	2	4	2
	BB	b_2	1	2	1

These relative frequencies are the expected relative frequencies arising from the selfing of the doubles heterozygote in the absence of linkage: for example, the selfing of *AaBb* or a_1b_1 gives *AaBB* or a_1b_2 in 2/16 of the cases.

Genotypic values will be denoted also by the symbol a_ib_j so that a_2b_1 denotes both the genotype *AABb* and the genotypic value of *AABb* inidviduals.

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Whether the genotype or genotypic value is meant will always be clear from the context. Let μ equal the mean of the genotypic values in the table, that is

$$\mu = \frac{(a_0 + 2a_1 + a_2)\ (b_0 + 2b_1 + b_2)}{16}$$

where the expression is to be expanded and the genotypic values inserted.

Now let

$$A_0 = \frac{(a_0b_0 + 2a_0b_1 + a_0b_2)}{4} - \mu$$

$$A_1 = \frac{(2a_1b_0 + 4a_1b_1 + 2a_1b_2)}{8} - \mu$$

$$A_2 = \frac{(a_2b_0 + 2a_2b_1 + a_2b_2)}{4} - \mu$$

and B_0, B_1 and B_2 can be expressed similarly. It is interesting to note that

$$A_0 + 2A_1 + A_2 = B_0 + 2B_1 + B_2 = 0.$$

The interaction deviation is

$$a_ib_j - \mu - A_i - B_j$$

and is therefore a deviation of a_ib_j from a model additive in loci effects. For example, the deviation for a_2b_1 is $a_2b_1 - \mu - A_2 - B_1$ which equals $\frac{1}{16}(-a_0 - 2a_1 + 3a_2)(-b_0 + 2b_1 - b_2)$. In general the interaction deviations can be written down formally by the multiplication of certain symbols. Note that formally

$$A_0 = a_0 \frac{(b_0 + 2b_1 + b_2)}{4} - \mu$$

$$= \left[a_0 - \frac{1}{4}(a_0 + 2a_1 + a_2)\right] \frac{(b_0 + 2b_1 + b_2)}{4}$$

$$= \frac{1}{16}(3a_0 - 2a_1 - a_2)\ (b_0 + 2b_1 + b_2).$$

Likewise

$$A_1 = \frac{1}{16}(-a_0 + 2a_1 - a_2)\ (b_0 + 2b_1 + b_2)$$

and

$$A_2 = \frac{1}{16}(-a_0 - 2a_1 + 3a_2)\ (b_0 + 2b_1 + b_2).$$

Similarly

$$B_0 = \frac{1}{16}(a_0 + 2a_1 + a_2)\ (3b_0 - 2b_1 - b_2)$$

$$B_1 = \frac{1}{16}(a_0 + 2a_1 + a_2)\ (-b_0 + 2b_1 - b_2)$$

$$B_2 = \frac{1}{16}(a_0 + 2a_1 + a_2)\ (-b_0 - 2b_1 + 3b_2).$$

We will now denote

$$\frac{1}{4}(3a_0 - 2a_1 - a_2) \text{ by } A_0, \quad \frac{1}{4}(3b_0 - 2b_1 - b_2) \text{ by } B_0,$$

$$\frac{1}{4}(-a_0 + 2a_1 - a_2) \text{ by } A_1, \quad \frac{1}{4}(-b_0 + 2b_1 - b_2) \text{ by } B_1,$$

$$\frac{1}{4}(-a_0 - 2a_1 + 3a_2) \text{ by } A_2 \text{ and } \frac{1}{4}(-b_0 - 2b_1 + 3b_2) \text{ by } B_2.$$

Then the interaction deviation for a_2b_1 can be denoted by A_2B_1 since this deviation is equal to

$$\frac{1}{4}(-a_0 - 2a_1 + 3a_2)\frac{1}{4}(-b_0 + 2b_1 - b_2) = \frac{1}{16}(-a_0 - 2a_1 + 3a_2)\ (-b_0 + 2b_1 - b_2)$$

as given previously. The same formulation holds for the other interaction deviations so that we have

$$a_ib_j = \mu + A_i + B_j + A_iB_j$$

where the symbol A_2, for example, means $\frac{1}{4}(-a_0 - 2a_1 + 3a_2)$ evaluated over all possibilities for unmentioned loci, that is over the b locus, B_1 means $\frac{1}{4}(-b_0 + 2b_1 - b_2)$ evaluated over unmentioned loci, that is over the a locus, and A_2B_1 means $\frac{1}{16}(-a_0 - 2a_1 + 3a_2)(-b_0 + 2b_1 - b_2)$ evaluated over all unmentioned loci, in this case none.

The model for the two locus case, given here, corresponds to the 2^2 factorial model for two factors n and p, say, in which

$$np = \mu + N + P + NP$$

(See KEMPTHORNE (1952, p. 235)).
In general the model is given by the following equation

$$a_ib_jc_k\text{----} = +\mu + A_i + B_j + A_iB_j + C_k + A_iC_k + B_jC_k + A_iB_jC_k + \quad .$$

where:

(1) the individual under consideration is at the ith (i = 0, 1 or 2) phase at locus *a*, jth (j = 0, 1 or 2) phase at locus *b*, kth (k = 0, 1 or 2) phase at locus *c* and so on. The phases 0, 1 and 2 indicate the number of dominant alleles at each locus.

(2) $a_ib_jc_k$---- = genotypic value for a character with any number of loci.

(3) μ = overall mean.

(4) $A_0 = \frac{1}{4}(3a_0 - 2a_1 - a_2)$ evaluated over all the possible combinations for all other loci, or the locus effect of locus *a* at phase zero.

(5) $A_1 = \frac{1}{4}(-a_0 + 2a_1 - a_2)$ evaluated over all the possible combinations for all other loci.

(6) $A_2 = \frac{1}{4}(-a_0 - 2a_1 + 3a_2)$ evaluated over all the possible combinations for all other loci.

(7) Similarly for B_j (j = 0, 1 or 2), C_k (k = 0, 1 or 2) and so on.

(8) A_iB_j = product of A_i and B_j (for all i, j and k) and so on, evaluated over all unmentioned loci.

(9) Similarly for A_iC_k, B_jC_k (for all i, j and k) and so on, evaluated over all unmentioned loci.

(10) $A_iB_jC_k$ = product of A_iB_j and C_k (for all i, j and k), evaluated over all unmentioned loci.

(11) Similarly, all other combinations are formed by products.

In order to validate the accuracy of the model we must show that the equation above is an identity. That is, we must prove that

$$a_ib_jc_k---- \equiv \mu + A_i + B_j + A_iB_j + C_k + A_iC_k + B_jC_k + A_iB_jC_k + \ldots.$$

We know that

$$\mu = \left(\frac{(a_0 + 2a_1 + a_2)}{4}\right)\left(\frac{(b_0 + 2b_1 + b_2)}{4}\right)\left(\frac{(c_0 + 2c_1 + c_2)}{4}\right)\ldots,$$

but let us make the notation:

$$\overline{A} = \frac{(a_0 + 2a_1 + a_2)}{4}$$

$$\overline{B} = \frac{(b_0 + 2b_1 + b_2)}{4}$$

$$\overline{C} = \frac{(c_0 + 2c_1 + c_2)}{4}$$

and so on.
It follows that

$$a_i = \overline{A} + A_i$$
$$b_j = \overline{B} + B_j$$
$$c_k = \overline{C} + C_k,$$

so we have to prove

$$(\overline{A} + A_i)\ (\overline{B} + B_j)\ (\overline{C} + C_k)\ldots$$
$$= \overline{ABC}\ldots + A_i\overline{BC}\ldots + \overline{A}B_j\overline{C}\ldots + A_iB_j\overline{C}\ldots$$
$$+ \overline{AB}C_k\ldots + A_i\overline{B}C_k\ldots + \overline{A}B_jC_k\ldots + A_iB_jC_k\ldots.$$

It is clear that the right-hand side of this equation is the expansion of the left-hand side, for we may first remove the factor $(\overline{A} + A_i)$ from the left-hand side, then the factor $(\overline{B} + B_j)$ and so on to exhaust completely the right-hand side. Hence it follows that

$$a_ib_jc_k\text{---} \equiv \mu + A_i + B_j + A_iB_j + C_k + A_iC_j + B_kC_j + A_iB_jC_k + \ldots.$$

A few of the desirable features of this model are the following:

1. There is additivity of the parameters.
2. The parameters have genetical meaning. This can be shown as follows:
   (a) The basic gene effect or additive genetic effect, at any locus r, say, $\frac{1}{2}(R_2 - R_0)$
   where R_2 is the effect of RR
   R_0 is the effect of rr.
   (b) The dominance effect at locus r is $\frac{1}{2}(2R_1 - R_2 - R_0)$, where R_1 is the effect of Rr and R_2 and R_0 are denoted as in (a) above.
   (c) The epistatic effect at locus r in phase i with locus s in phase j is R_iS_j where i, j = 0, 1 or 2. In a similar manner we may express the epistatic effect of any number of loci in all phases.
3. The model applies for any number of loci.
4. The model is symmetric. This can be seen when we look at the original definitions of A_0 and A_2. It has been given for the two locus cases that

$$A_0 = \frac{(a_0b_0 + 2a_0b_1 + a_0b_2)}{4} - \mu$$

and

$$A_2 = \frac{(a_2b_0 + 2a_2b_1 + a_2b_2)}{4} - \mu.$$

   If we replace a_0 by a_2 in the expression for A_0 we get A_2, and if we replace a_2 by a_0 in the expression for A_2 we get A_0. Hence the factorial model is symmetric.
5. The model is flexible in the sense that as more assumptions are made parameters are merely dropped from the model and the model retains, without further computations, its additive structure. For example, if there is no epistasis, that is no interaction, then the model becomes

   $$a_ib_jc_k\text{----} = \mu + A_i + B_j + C_k + \text{----}, \text{ and}$$

   the model is additive in the locus effects.

THE APPLICATION OF THE FACTORIAL MODEL TO SELF-FERTILIZATION

The model was applied to populations obtained by successively selfing from an n-loci heterozygous individual. Since the model is symmetric with respect to loci, we need only consider the p-factor interactions arising from one set of the p loci. We consider the interactions arising from the first p loci for which the letters are say a, b, c, . . . , p. The total genotypic array in the mth generation of selfing is

$$\left[\frac{1}{2}(1-q)\,aa + qAa + \frac{1}{2}(1-q)\,AA\right]\left[\frac{1}{2}(1-q)\,bb + qBb + \frac{1}{2}(1-q)\,BB\right]\ldots.$$

$$\ldots\left[\frac{1}{2}(1-q)\,pp + qPp + \frac{1}{2}(1-q)\,PP\right] \text{ and so on, where q is equal to } \frac{1}{2^m}.$$

To evaluate the mean genotypic value we expand the above product and insert the genotypic value as given by the model for each genotype. We consider only the p-factor interaction contributions from loci $a, b, c,$, p. The mean contribution is obtained by expanding

$$\left[\frac{1}{2}(1-q)\,A_0 + qA_1 + \frac{1}{2}(1-q)\,A_2\right]\left[\frac{1}{2}(1-q)\,B_0 + qB_1 + \frac{1}{2}(1-q)\,B_2\right]\ldots.$$

$$\ldots\left[\frac{1}{2}(1-q)\,P_0 + qP_1 + \frac{1}{2}(1-q)\,P_2\right].$$

From the method of construction of the model if X, Y, Z---- and so on are symbols such as B_1, C_2 and so on, we know that

$$A_0XYZ\text{----} + 2A_1XYZ\text{----} + A_2XYZ\text{----} = 0.$$

Hence $(A_0 + A_2)XYZ\text{----} = -2A_1XYZ\text{----}$. We may therefore substitute for $(A_0 + A_2)$ in the first factor by means of this expression, likewise in the other factors to get

$$\prod_{\text{first p loci}}\left[qA_1 + \left(\frac{1-q}{2}\right)(A_0 + A_2)\right] =$$

$$\prod_{\text{first p loci}}\left[qA_1 - (1-q)\,A_1\right] =$$

$$\prod_{\text{first p loci}}(2q-1)\,A_1 =$$

$$(2q-1)^p\,A_1B_1C_1\ldots P_1,$$

where q is the frequency of a heterozygous allele after m generations of selfing or is $\frac{1}{2^m}$.

Substituting $\frac{1}{2^m}$ for q in the last expression above we obtain

$$\left(\frac{1}{2^{m-1}} - 1\right)^p A_1B_1C_1 \ldots P_1.$$

We now sum over all possible p loci and let

$$\beta_p = \sum_{\substack{\text{all sets} \\ \text{of p loci}}} A_1B_1C_1 \ldots P_1.$$

We find that the p-factor interaction contribution is

$$\left(\frac{1}{2^{m-1}} - 1\right)^p \beta_p.$$

This proof also holds for the interaction of the fixed loci with any p-factor interaction of the variable loci.

It follows that the mean genotypic value of the population arising from selfing a completely heterozygous individual for m generations can be summarized as:

$$\overline{F}_{m+1} = K_1 \sum_{p=1}^{n} \left(\frac{1}{2^{m-1}} - 1\right)^p \beta_p$$

where $\overline{F}_{m+1}$ = the mean genotypic value of the population obtained after m generations of self-fertilization of any given genotype,

$K_1 = \mu$, the overall mean, plus the loci effects of the fixed loci, and

β_p = the p-factor interaction of the variable loci plus the interaction of the p-factor interactions with the fixed loci.

Hence

$$\overline{F}_1 = K_1 + \sum_{p=1}^{n} \beta_p, \text{ where } \overline{F}_1 \text{ is the original heterozygous parent,}$$

$$\overline{F}_2 = K_1$$

$$\overline{F}_3 = K_1 + \sum_{p=1}^{n} \left(-\frac{1}{2}\right)^p \beta_p.$$

$$\overline{F}_4 = K_1 + \sum_{p=1}^{n} \left(-\frac{3}{4}\right)^p \beta_p$$

$$\vdots$$

$$\overline{F}_\infty = K_1 + \sum_{p=1}^{n} (-1)^p \beta_p.$$

The algebraic value of the coefficient of the locus effects of the variable loci and the interaction of each variable locus with the fixed loci (β_1) decreases as the number of generations of self-fertilization increases. The coefficient of the two-factor interactions of the variable loci and the interaction of every possible combination of two of the variable loci with the fixed loci (β_2) decreases to the $\overline{F}_3$ and increases slowly as the number of generations of self-fertilization increases from $\overline{F}_3$. The coefficient of β_3 acts as the coefficient of β_1; however, the algebraic value of the coefficient of β_3 is much smaller than the coefficient of β_1 beyond the $\overline{F}_2$.

To examine explanations for inbreeding depression, we shall suppose that interactions involving three loci or more are trivial. This corresponds to FISHER'S (1918) statement, "... it is very improbable that any statistical effect, of a nature other than that which we are considering is actually produced by more complex somatic connections," prior to which he had explained dual epistacy. Then we have

$$F_1 = K_1 + \beta_1 + \beta_2$$

$$\overline{F}_2 = K_1$$

$$\overline{F}_3 = K_1 - \frac{1}{2}\beta_1 + \frac{1}{4}\beta_2$$

$$\overline{F}_4 = K_1 - \frac{3}{4}\beta_1 + \frac{9}{16}\beta_2$$

$$\overline{F}_5 = K_1 - \frac{7}{8}\beta_1 + \frac{49}{64}\beta_2$$

and so on.

There is no particular reason for β_1 to be negative or positive. If β_1 is negative and we assume the interactions are negligible, we should get no inbreeding depression. In fact we would expect a slight increase rather than a decrease in the mean genotypic value with inbreeding. If β_2 were negative and large enough to influence the genotypic value, the inbreeding depression could be explained by epistasis. Hence an explanation of inbreeding depression must, it appears, give attention to epistasis as well as to loci effects.

It should perhaps be restated that the development given here is based on the complete absence of selection, including of course the absence of lethals, on equal viability of all genotypes and on gene frequency of .5. The extent to which these contribute to inbreeding depression cannot be ascertained from the type of data we are considering.

Example:

KHAMBANONDA (1948) presented the data given in table 1. The mean squares for all generations are given in KHAMBANONDA'S thesis, but are not reproduced here. These mean squares were used to obtain variances of estimates given in table 2. Following the statistical procedure given by KEMP-

TABLE 1

Means of selfed generations.

Generation	Number of observations	Length	Width	Shape = (length/width)	
				Arithmetic	Logarithmic
$\bar{F}_1$	300	51.3	25.1	2.04	.309
$\bar{F}_2$	900	48.9	25.0	2.10	.280
$\bar{F}_3$	898	51.5	23.4	2.42	.330
F_4	897	50.1	23.2	2.40	.325

Generation	Green fruit weight		Dry fruit weight	
	Arithmetic	Logarithmic	Arithmetic	Logarithmic
$\bar{F}_1$	59.6	1.771	10.1	1.000
$\bar{F}_2$	61.7	1.758	9.6	0.963
$\bar{F}_3$	56.3	1.694	9.1	0.930
F_4	53.7	1.673	8.8	0.915

THORNE (1952), chapter five, the least squares estimates of K_1, β_1 and β_2 and the standard errors of these estimates were obtained and are presented in table 2. From the tables the following conclusions are drawn:

(1) For length and shape of fruit, $\hat{\beta}_1$ is negative and $\hat{\beta}_2$ is positive. From our knowledge of the model we should expect no apparent depression. The data substantiate this view. For width and fruit weight $\hat{\beta}_1$ is positive and $\hat{\beta}_2$ is negative, except for dry fruit weight. We expect a decided depression, and the data uphold this view also.

(2) If we assume the sampling distribution of $\hat{\beta}_2$ to be normal and all tests to be independent, we would reject at the 5 percent level the hypothesis that $\hat{\beta}_2$ is zero in all cases. This tells us that we reject the hypothesis that there is no epistasis.

TABLE 2

Estimates of parameters and their standard errors.

Character	$\hat{K}_1$	(s.e. $\hat{K}_1$)	$\hat{\beta}_1$	(s.e. $\hat{\beta}_1$)	$\hat{\beta}_2$	(s.e. $\hat{\beta}_2$)
Length of fruit	49.571	±.0484	− .2804	±.0475	1.9269	± .0838
Width of fruit	24.826	±.0165	1.5211	±.0164	−1.2172	± .0288
Shape (arithmetic)	2.146	±.0316	− .2816	±.0312	.1695	± .0548
Shape (logarithmic)	0.288	±.0058	− .0268	±.0057	.0463	± .0101
Green fruit weight (arithmetic)	61.403	±.7748	5.3804	±.7686	−7.1269	±1.3477
Green fruit weight (logarithmic)	1.754	±.0054	.0731	±.0058	− .0548	± .0097
Dry fruit weight (arithmetic)	9.600	±.0861	.8252	±.0879	− .3226	± .1516
Dry fruit weight (logarithmic)	0.962	±.0040	.0530	±.0043	− .0149	± .0072

TWO PARENTS

The model was applied to populations which arise by crossing two inbred lines and subsequent crossing and selfing. The loci fall into groups within which the loci have identical phase relationships to the parents. We may describe the parents as

	Loci group			
Parent	1	2	3	4
P_1	R	D	R	D
P_2	R	R	D	D

where R and D are equivalent to phases 0 and 2 respectively. We also label phase 1 as H. Since loci groups one and four are always homozygous and fixed regardless of the cross, we need not use them to describe any genotypic value. also loci group three which contains variable loci is a reflection of loci group two, in the sense that phase D is a reflection of phase R and phase H is a reflection of itself. Hence we may specify completely any genotype by displaying only loci group two for that genotype. As illustrations of the notation, the loci group two of the F_1 mean is $D \times R = H$ and loci group two of the F_2 mean is $H \times H = \frac{1}{4}R + \frac{1}{2}H + \frac{1}{4}D$.

The following list includes the representation of the parents, crosses made by random mating and selfed populations obtained by crossing and then selfing. (The letter *S* will be used to denote selfed populations, for example, $(F_2)S = F_{2\cdot}$):

Population	*Loci Group Two*
P_1	D
P_2	R
$(P_1)(P_2) = F_1$	H
$(F_1)(F_1) = F_2$	$\frac{1}{4}R, \frac{1}{2}H, \frac{1}{4}D$
$(P_1)(F_1)$	$\frac{1}{2}H, \frac{1}{2}D$
$(P_2)(F_1)$	$\frac{1}{2}R, \frac{1}{2}H$
$(P_1)[(P_1)(F_1)]$	$\frac{1}{4}H, \frac{3}{4}D$
$(P_2)[(P_1)(F_1)]$	$\frac{1}{4}R, \frac{3}{4}H$
$(P_1)[(P_2)(F_1)]$	$\frac{3}{4}H, \frac{1}{4}D$

$(P_2)\,[(P_2)\,(F_1)]$	$\frac{3}{4}R, \frac{1}{4}H$
$(F_1)\,[(P_1)\,(F_1)]$	$\frac{1}{8}R, \frac{1}{2}H, \frac{3}{8}D$
$(F_1)\,[(P_2)\,(F_1)]$	$\frac{3}{8}R, \frac{1}{2}H, \frac{1}{8}D$
$(F_1)S = F_2$	$\frac{1}{4}R, \frac{1}{2}H, \frac{1}{4}D$
$(F_2)S = F_3$	$\frac{3}{8}R, \frac{1}{4}H, \frac{3}{8}D$
$(F_3)S = F_4$	$\frac{7}{16}R, \frac{1}{8}H, \frac{7}{16}D$
$[(P_1)\,(F_1)]S$	$\frac{1}{8}R, \frac{1}{4}H, \frac{5}{8}D$
$[(P_2)\,(F_1)]S$	$\frac{5}{8}R, \frac{1}{4}H, \frac{1}{8}D$

We shall now obtain the mean genotypic value of a population represented by

$$pR + qH + rD.$$

Apart from fixed loci the genotypic array is

$$\prod_{\substack{\text{loci of}\\ \text{group two}}} (pR + qH + rD) \qquad \prod_{\substack{\text{loci of}\\ \text{group three}}} (rR + qH + pD).$$

Consider in order the loci effects, two-factor interactions and so on which occur when this array is expanded and the factorial model is inserted in place of the genotype.

The total effect contribution is

$$\sum_{\substack{\text{fixed}\\ \text{loci}}} (\text{effects}) + \sum_{\substack{\text{loci of}\\ \text{group two}}} (pA_0 + qA_1 + rA_2) + \sum_{\substack{\text{loci of}\\ \text{group three}}} (rB_0 + qB_1 + pB_2)$$

The two-factor interactions are of three types (1) interactions between variable loci, (2) interactions of a variable locus with a fixed locus and (3) interactions of two fixed loci. As regards (3), we shall get a certain fixed quantity. As regards (2), suppose a_0 refers to a fixed locus then we obtain

$$\sum_{\substack{\text{loci of}\\ \text{group two}}} (pa_0A_0 + qa_0A_1 + ra_0A_2)$$

with similar expressions for all loci of group two and group three. As regards (1) we have three types (a) interaction of two loci in group two, (b) interaction of two loci in group three, (c) interaction of one locus in group two with one locus in group three.

When we combine these terms together with the loci effects and ignore interactions of more than two loci we obtain the expression

$$\begin{aligned}
K_2 &+ p(\underset{2}{\Sigma}A_0 + \underset{3}{\Sigma}B_2) + q(\underset{2}{\Sigma}A_1 + \underset{3}{\Sigma}B_1) + r(\underset{2}{\Sigma}A_2 + \underset{3}{\Sigma}B_0) \\
&+ p^2\{\underset{2}{\Sigma}A_0A_0' + \underset{3}{\Sigma}B_2B_2' + \underset{2,3}{\Sigma}A_0B_2\} \\
&+ q^2\{\underset{2}{\Sigma}A_1A_1' + \underset{3}{\Sigma}B_1B_1' + \underset{2,3}{\Sigma}A_1B_1\} \\
&+ r^2\{\underset{2}{\Sigma}A_2A_2' + \underset{3}{\Sigma}B_0B_0' + \underset{2,3}{\Sigma}A_2B_0\} \\
&+ pq\{2\underset{2}{\Sigma}A_0A_1' + 2\underset{3}{\Sigma}B_2B_1' + \underset{2,3}{\Sigma}A_0B_1 + \underset{2,3}{\Sigma}A_1B_2\} \\
&+ pr\{2\underset{2}{\Sigma}A_0A_2' + 2\underset{3}{\Sigma}B_0B_2' + \underset{2,3}{\Sigma}A_0B_0 + \underset{2,3}{\Sigma}A_2B_2\} \\
&+ qr\{2\underset{2}{\Sigma}A_1A_2' + 2\underset{3}{\Sigma}B_0B_1' + \underset{2,3}{\Sigma}A_1B_0 + \underset{2,3}{\Sigma}A_2B_1\}
\end{aligned}$$

where, for example,

$\underset{2}{\Sigma}A_0$ = the sum of the locus effects for loci in the R phase in group two

$\underset{2}{\Sigma}A_0A_0'$ = the sum of interaction contributions from two loci in group two, both of which are in the R phase

$\underset{2}{\Sigma}A_0A_2'$ = the sum of interaction contributions from two loci in group two, one of which is in the R phase and the other of which is in the D phase

$\underset{2,3}{\Sigma}A_0B_1$ = the sum of interaction contributions arising from two loci, one of which is in group two and is in the R phase, and the other of which is in group three and is in the H phase, and where all effects and interactions are defined with respect to the variable loci only.

There are ten parameters in this expression; however, we can reduce the number of parameters to six when all the restrictions of the type

$$(A_0 + 2A_1 + A_2) = (B_0 + 2B_1 + B_2) = 0$$

are used. To simplify the notation we will let

$$E = (\underset{2}{\Sigma}A_1 + \underset{3}{\Sigma}B_1),$$

$$F = (\underset{2}{\Sigma}A_2 + \underset{3}{\Sigma}B_0),$$

$$G = (\underset{2}{\Sigma}A_1A_1' + \underset{3}{\Sigma}B_1B_1' + \underset{2,3}{\Sigma}A_1B_1),$$

$$L = (2\underset{2}{\Sigma}A_1A_2' + 2\underset{3}{\Sigma}B_1B_0' + \underset{2,3}{\Sigma}A_1B_0 + \underset{2,3}{\Sigma}A_2B_1),$$

and $$M = (\underset{2}{\Sigma}A_2A_2' + \underset{3}{\Sigma}B_0B_0' + \underset{2,3}{\Sigma}A_2B_0).$$

Due to the construction of the model

$$(\underset{2}{\Sigma}A_0 + \underset{3}{\Sigma}B_2) = -(2E + F),$$

$$(\sum_{2} A_1A_0' + \sum_{2} A_0A_1' + \sum_{3} B_1B_2' + \sum_{2,3} A_1B_2 + \sum_{2,3} A_0B_1) = -(4G + L)$$

and $$(\sum_{2} A_0A_0' + \sum_{3} B_2B_2' + \sum_{2,3} A_0B_2) = (4G + 2L + M).$$

This specifies all locus effects and possible interactions of two loci. Hence we may describe the six parameters as,

K_2 which is the contribution due to the overall mean plus the locus effects and interactions of the fixed loci,

E and F which are the contributions due to the variable effects plus the interaction of these locus effects with the fixed loci, and

G, L and M which are the contributions due to the interaction of all possible pairs of variable loci plus the interaction of these interactions with the fixed loci.

It should be emphasized that due to the construction of the model the coefficient of G is the square of the coefficient of E, the coefficient of L is equal to the product of the coefficient of E and of the coefficient of F, and the coefficient of M is the square of the coefficient of F Hence after we obtain the coefficients of E and F, we can write down the coefficients of G, L and M immediately.

Using these definitions of the locus effects and interactions and the frequencies of the various phases of the loci in group two we can write the factorial representation of the means of the genotypic values of the populations listed previously as follows:

$$P_1 = K_2 + F + M$$
$$P_2 = K_2 - 2E - F + 4G + 2L + M$$
$$F_1 = K_2 + E + G$$
$$F_2 = K_2$$
$$(P_1)(F_1) = K_2 + \frac{1}{2}E + \frac{1}{2}F + \frac{1}{4}G + \frac{1}{4}L + \frac{1}{4}M$$
$$(P_2)(F_1) = K_2 - \frac{1}{2}E - \frac{1}{2}F + \frac{1}{4}G + \frac{1}{4}L + \frac{1}{4}M$$
$$(P_1)[(P_1)(F_1)] = K_2 + \frac{1}{4}E + \frac{3}{4}F + \frac{1}{16}G + \frac{3}{16}L + \frac{9}{16}M$$
$$(P_2)[(P_1)(F_1)] = K_2 + \frac{1}{4}E - \frac{1}{4}F + \frac{1}{16}G - \frac{1}{16}L + \frac{1}{16}M$$
$$(P_1)[(P_2)(F_1)] = K_2 + \frac{3}{4}E + \frac{1}{4}F + \frac{9}{16}G + \frac{3}{16}L + \frac{1}{16}M$$
$$(P_2)[(P_2)(F_1)] = K_2 - \frac{5}{4}E - \frac{3}{4}F + \frac{25}{16}G + \frac{15}{16}L + \frac{9}{16}M$$
$$(F_1)[(P_1)(F_1)] = K_2 + \frac{1}{4}E + \frac{1}{4}F + \frac{1}{16}G + \frac{1}{16}L + \frac{1}{16}M$$
$$(F_1)[(P_2)(F_1)] = K_2 - \frac{1}{4}E - \frac{1}{4}F + \frac{1}{16}G + \frac{1}{16}L + \frac{1}{16}M$$

$$(F_1)S = F_2 = K_2$$

$$F_3 = K_2 - \frac{1}{2}E + \frac{1}{4}G$$

$$F_4 = K_2 - \frac{3}{4}E + \frac{9}{16}G$$

$$[(P_1)(F_1)]S = K_2 + \frac{1}{2}F + \frac{1}{4}M$$

$$[(P_2)(F_1)]S = K_2 - E - \frac{1}{2}F + G + \frac{1}{2}L + \frac{1}{4}M$$

With the parents and the crosses very often used in experimentation, that is, F_1, F_2 and the two backcrosses we obtained the following estimates of the six parameters:

$$\hat{K}_2 = F_2$$

$$\hat{E} = -\frac{1}{4}P_1 - \frac{1}{4}P_2 + \frac{1}{2}F_1 - 2F_2 + (P_1)(F_1) + (P_2)(F_1)$$

$$\hat{F} = \frac{1}{4}P_1 + \frac{1}{4}P_2 - \frac{1}{2}F_1 + 2F_2 - 2(P_2)(F_1)$$

$$\hat{G} = \frac{1}{4}P_1 + \frac{1}{4}P_2 + \frac{1}{2}F_1 + F_2 - (P_1)(F_1) - (P_2)(F_1)$$

$$\hat{L} = -P_1 - F_1 - 2F_2 + 3(P_1)(F_1) + (P_2)(F_1)$$

$$\hat{M} = \frac{3}{4}P_1 - \frac{1}{4}P_2 + \frac{1}{2}F_1 - 3F_2 + 2(P_2)(F_1)$$

The variances of these estimates are obtained in the usual manner, for example,

$$\sigma^2_{\hat{L}} = \sigma^2_{P_1} + \sigma^2_{F_1} + 4\sigma^2_{F_2} + 9\sigma^2_{(P_1)(F_1)} + \sigma^2_{(P_2)(F_1)}.$$

In ANDERSON (1953), estimates are given for the various parameters using data furnished by STRINGFIELD (1949) on three characters in corn, number of days to midsilk, ear node height and yield of corn. Comparisons of magnitudes between locus effects and epistatic effects are made and for all three characters some of the estimates of epistasis are of the same magnitude or larger than the estimates of locus effects. Hence, it was concluded that the epistatic contributions are important in the particular material investigated.

It is of interest to investigate the scaling tests given by MATHER (1949). The test, using A, B and C, given by MATHER tests the additiveness of the genic effects regardless of the presence of dominance or linkages. This seems to imply, and it is certainly accepted by many, that this is a test for absence of epistasis. If the interaction of three or more loci is known to be zero then this

is a test of the two-factor interactions being zero. However, if the interactions of three loci only are not zero, this is not a test for the absence of epistasis. The reason for this may be summarized by expressing A, B and C in terms of the factorial model as follows:

$$A = -\frac{1}{2}G + \frac{1}{2}L - \frac{1}{2}M + \frac{1}{4}\alpha + \frac{1}{4}\beta + \frac{1}{4}\gamma + \frac{1}{4}\delta$$

$$B = -\frac{9}{2}G - \frac{3}{2}L - \frac{1}{2}M + \frac{27}{4}\alpha + \frac{3}{4}\beta + \frac{15}{4}\gamma + \frac{7}{4}\delta$$

$$C = -6G - 2L - 2M + 6\alpha + 4\gamma + 2\delta,$$

where α, β, γ and δ are the three factor interaction parameters. Hence if $A = B = C = 0$, it does not necessarily follow that the parameters are zero.

We have not discussed in this study the variances of populations although it may be expected that such an examination would yield further information on the general problem of the importance of epistasis.

OTHER CASES

Using the very desirable characteristic of the model, symmetry, we were able to investigate the three-parent case as thoroughly as the two-parent case and the four-parent case when we assumed there was no epistasis. The description of the technique employed is explained in ANDERSON (1953).

SUMMARY

The factorial gene model, which is an adaptation of the factorial model used in the design of experiments, is developed and applied to problems in quantitative inheritance. The model is based on comparisons in the population of all possible genotypes in which heterozygotes are given twice the weight of homozygotes and is therefore appropriate for populations in which gene frequency is 0.5. As a result the model for the genotypic value of an individual represented by $a_ib_jc_k \ldots$, that is an individual in which the a locus is in the i^{th} phase, the b locus is in the j^{th} phase and so on, is

$$\mu + A_i + B_j + A_iB_j + C_k + A_iC_k + B_jC_k + A_iB_jC_k + \ldots$$

where μ represents the sum of constant effects,

A_i, B_j, C_k and so on represent loci effects,
A_iB_j, A_iC_k, B_jC_k, $A_iB_jC_k$ and so on represent interactions of loci or the epistatic effects.

The model has the following desirable characteristics:

(1) additivity of the parameters,
(2) parameters which have genetic interpretation,
(3) applicability to a genotypic value for any number of loci,
(4) symmetry with respect to the homozygous phases at each locus, and
(5) flexibility of the model with respect to increasing assumptions.

In this paper we assumed that linkages and lethal genes were absent and that viability was constant for all genotypes.

The factorial model was applied to the means of populations obtained by successively selfing from a single individual. The general formula for the mean genotypic value of the population produced by m generations of selfing is

$$F_{m+1} = K_1 + \sum_{p=1}^{n} \left(\frac{1}{2^{m-1}} - 1\right)^p \beta_p$$

where β_p is the contribution of the p-factor interactions and K_1 is the overall effect of the loci which are homozygous in the original individual.

The model was applied to data, KHAMBANONDA (1948), and it was concluded that in his material the epistatic contributions may be an important cause of inbreeding depression. Also, it was found in all cases that the epistatic contribution differed significantly, at the 5% level, from zero.

The model was applied to some of the populations which arise by crossing two inbred lines and subsequent crossing and selfing. Locus effects and epistatic parameters were estimated and the results were applied to data given by STRINGFIELD (1949). It was found that for the characteristics considered the epistatic components were an important part of the observed mean genotypic values. The model enables examination of the scaling tests described by MATHER (1949) and these are not necessarily tests for absence of epistasis.

Extensions to the cases of three and four parents are straightforward but tedious and are not presented here.

LITERATURE CITED

ANDERSON, V. L., 1953 A model for the study of quantitative inheritance. Unpublished Ph.D. thesis. Ames, Iowa, Iowa State College Library.

FISHER, R. A., 1918 The correlation between relatives on the supposition of Mendelian inheritance. Trans. Royal Soc. Edin. **52**: 399–433.

FISHER, R. A., F. R. IMMER and O. TEDIN, 1932 The genetical interpretation of statistics of the third degree in the study of quantitative inheritance. Genetics **17**: 107–123.

GRIFFING, J. B., 1950 Analysis of quantitative gene action by constant parent regressions and related techniques. Genetics **35**: 303–321.

KEMPTHORNE, O., 1952 The Design and Analysis of Experiments. New York, John Wiley and Sons, Inc.

KHAMBANONDA, I., 1948 Polygenic inheritance of fruit size in red pepper. Unpublished Ph.D. thesis. Ames, Iowa, Iowa State College Library.

MATHER, K., 1949 Biometrical Genetics. Dover Publications, Inc.

STRINGFIELD, G. H., 1949 Senior Agronomist, Agricultural Experiment Station, Wooster, Ohio. (Data obtained by personal communication.)

RATES OF SPONTANEOUS MUTATION IN THE SECOND CHROMOSOMES OF THE SIBLING SPECIES, DROSOPHILA PSEUDOOBSCURA AND DROSOPHILA PERSIMILIS[1]

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WE have recently reported the results of a comparison of the spontaneous mutation rates for recessive lethals and semilethals in the second and third chromosomes (autosomes) of two neotropical species of Drosophila, namely *D. willistoni* and *D. prosaltans* (DOBZHANSKY, SPASSKY and SPASSKY 1952). The chromosomes of these species are known to be homologous, since they contain mostly the same gene loci. Although the mutation rates in both species varied from strain to strain, and probably from locality to locality, the material at hand indicated that lethal and semilethal mutations are roughly twice as frequent in *D. willistoni* as in *D. prosaltans*. This may possibly be correlated with the fact that *D. willistoni* is a very common and ecologically highly versatile species, while *D. prosaltans* is much less common, spotty in its distribution, and ecologically apparently more specialized.

The only other species with which the *D. willistoni* and *D. prosaltans* may be compared with respect to their mutation rates is *D. melanogaster*, since the second chromosomes of these three species are supposed to be homologous. The mutation rates recorded in the literature for the second chromosome of *D. melanogaster* are about as high as they are in *D. willistoni*. Since, however, *D. melanogaster* is, in the temperate zone, an introduced species associated with man, comparisons of its ecological status with that of the other species are unreliable. The only other species for which spontaneous mutation rates have been recorded is *D. pseudoobscura*. The rate of origin of recessive lethals and semilethals has been studied for the third chromosome of the last named species (DOBZHANSKY and WRIGHT 1941). *D. pseudoobscura* is the commonest species of the genus in most forested localities in the western United States, as well as in parts of Mexico and Guatemala. Its ecological status is about like that of *D. willistoni* in the American Tropics. It happens, however, that the third chromosome of *D. pseudoobscura* is homologous to only a part of the second chromosome of *D. willistoni* and *D. prosaltans*, making direct comparison difficult (SPASSKY and DOBZHANSKY 1950, see also figure 1). However, the acrocentric third chromosomes of *D. willistoni* and *D. prosaltans* are in all probability homologous to the acrocentric second chromosomes of *D.*

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pseudoobscura and *D. persimilis* (fig. 1). *D. persimilis* is a sibling species closely related to *D. pseudoobscura,* and referred to in the older genetic literature under the confusing name of " Race B."

Accordingly, we have undertaken experiments to estimate the rates of origin of recessive lethal and semilethal mutations in the second chromosomes of *D. pseudoobscura* and *D. persimilis.* The resulting data permit comparisons of the spontaneous mutation rates in five species (including *D. melanogaster*). The species belong to the same subgenus (Sophophora), and the homologies of their chromosomes are known with a reasonable degree of assurance.

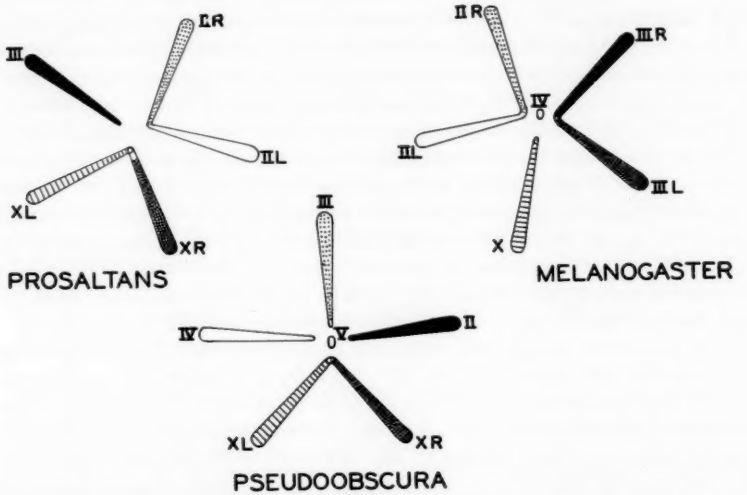

FIGURE 1.—The chromosome homologies in *Drosophila prosaltans* and *D. willistoni* (upper left), *D. melanogaster* (upper right), and *D. pseudoobscura* and *D. persimilis* (below). The homologous portions of the chromosomes are shown by similar shading; similarly numbered chromosomes are not necessarily homologous.

THE EXPERIMENTAL PROCEDURE

Collections of *D. pseudoobscura* and *D. persimilis* were made during the summer of 1951 in the Sierra Nevada of California, at Mather and at Porcupine Flats, in the Yosemite National Park region. The flies were shipped to New York, where lines were established starting from single females fertilized in nature by one or more males. Twelve lines of each species were chosen at random for the experiments, as shown in the leftmost column of table 1. The experiments were started during the winter 1951–1952. Since IVES (1950) and LEVINE and IVES (1953) observed changes in the mutation rates in strains kept in the laboratory, it is desirable to start experiments with fresh strains. The lines were known to have been free of lethals or semilethals at the beginning of the experiment, when standard tests were carried out (DOB-

ZHANSKY and SPASSKY 1953, the test strains having the Mather genetic background).

From each line 15–20 males were chosen and crossed individually to females homozygous for the recessive second-chromosome mutant glass (for *D. pseudoobscura*) or cardinal (for *D. persimilis*). In every subsequent generation, males from each strain, heterozygous for glass or for cardinal respectively, were taken and backcrossed to glass or to cardinal females. This was continued for 20 consecutive generations (18 generations for the strains derived from

TABLE 1

Mutations observed in the second chromosomes of Drosophila pseudoobscura (above) and Drosophila persimilis (below).

Origin		Number of strains	Chromosome-generations	Lethals	Semilethals	Visibles
Mather	407	17	340	3	2	....
"	409	19	380	....	1	....
"	413	17	340	....	....	....
"	419	18	360	2	....	....
"	430	19	342	....	....	1
"	466	13	260	1	1	....
"	478	19	380	2	2	....
"	497	17	340	3	....	....
"	499	17	340	3	3	....
"	506	17	340	5	2	....
"	519	19	380	2	3	....
Porcupine	1102	14	280	2	....	....
pseudoobscura		206	4082	23	14	1
Mather	105	16	320	4	1	....
"	404	17	340	7	....	....
"	406	18	360	5	1	....
"	412	16	320	3	1	1
"	422	19	380	6	....	....
"	476	16	320	3	2	....
"	501	17	340	8	....	....
"	521	18	360	4	....	....
"	535	18	360	5	1	....
Porcupine	1016	18	360	....	1	1
"	1055	18	360	5	1	....
persimilis		191	3820	50	8	2

Mather-430, see table 1). Some of the strains were lost in the process, but 206 *D. pseudoobscura* and 191 *D. persimilis* strains were successfully maintained (table 1). For a diagram showing an analogous experimental procedure, see DOBZHANSKY, SPASSKY and SPASSKY 1952.

In these crosses, the second chromosomes of Mather (or Porcupine) origin are transmitted from generation to generation through the male line. In every generation they are exposed to the risk of acquiring recessive mutants. Since these recessive mutants cannot manifest themselves in heterozygous condition, they may accumulate in the chromosomes. Mutants which are in-

completely recessive, and which reduce the fitness of the heterozygotes, may, of course, be lost in the process, but mutants of this kind are of limited importance for our present purpose (since they are rare in natural populations). Multiplying the numbers of the strains by the numbers of generations during which they were maintained, one obtains the numbers of chromosome-generations of exposure of the chromosomes to the risk of acquiring mutations (table 1).

The concluding part of the experiments consists of putting the second chromosomes in each strain through a series of crosses (schematically represented in figure 1 of the DOBZHANSKY, SPASSKY and SPASSKY, 1952, article). In *D. pseudoobscura,* the wild/glass males were outcrossed to females carrying a chromosome with the dominant gene Bare, the recessive glass, and an inversion which eliminates most of the crossing over (DOBZHANSKY and SPASSKY 1953). Bare non-glass females and males are selected in the next generation and inbred. One-third of the flies in the progeny must carry in duplicate the second chromosome which it is desired to test. If this chromosome now carries a recessive lethal gene, the homozygous class fails to appear. A semilethal or a visible recessive mutant is detected by a decrease of the number of the homozygotes, or by presence of morphological abnormalities respectively.

In *D. persimilis,* the males from the experimental strains are crossed to females carrying the dominant gene Delta, the recessive cardinal, and an inversion which suppresses all but a fraction of one percent of crossing over between Delta and cardinal. In the next generation, Delta non-cardinal females and males are selected and inbred; in the progeny, one-third of the flies (non-Delta) should carry in duplicate the second chromosomes to be tested. Lethals and semilethals can be detected by the absence, or by rarity, of the non-Delta class of flies.

In most cases, a cursory inspection under a binocular microscope of the flies produced in the test generation suffices to decide whether a lethal or an extreme semilethal (which causes death of most homozygotes) is or is not present in a given culture. Cultures in which such mutants were suspected were preserved, and complete counts of the flies hatching in them were made (the total of flies being usually more than 100). The possibility that some less extreme semilethals were missed cannot be excluded; since, however, this class of semilethals is relatively rare (see DOBZHANSKY and SPASSKY 1953, and other work), the error incurred by their possible loss is not considerable.

The numbers of strains in which mutations have been detected are listed in table 1. In all, 37 strains of *D. pseudoobscura* and 58 strains of *D. persimilis* carried recessive lethals or semilethals. One strain of *D. pseudoobscura* and two of *D. persimilis* had recessive mutants in their second chromosomes which gave rise to visible morphological abnormalities (one of these visibles acted also as a semilethal, so that it is listed twice in table 1).

All the experiments were carried at room temperatures, except that during

the hot spells in summer the cultures were kept in a constant temperature room at 25°C. The average temperature during the period of accumulation of lethals was probably in the neighborhood of 24°C. The experiments on *D. willistoni* and *D. prosaltans* were carried out under similar conditions (DOBZHANSKY, SPASSKY and SPASSKY 1952).

THE MUTATION RATES

The technique used to detect the lethals and semilethals does not distinguish between chromosomes which carried one lethal, and those which had two or more lethals. The actual number of lethal mutants per strain (m) may be computed from the Poisson formula as $m = \ln X - \ln Z$, where X is the number of strains tested, and Z the number of strains free of lethals and semilethals (cf. DOBZHANSKY, SPASSKY and SPASSKY 1952, page 655). The corrected frequencies of the lethal and semilethal mutations in the second chromosomes of *D. pseudoobscura* and *D. persimilis* (according to the data in table 1) and in the homologous third chromosomes of *D. willistoni* and *D. prosaltans*

TABLE 2

Corrected frequencies of lethal and semilethal mutations in Drosophila pseudoobscura and D. persimilis, and in the homologous chromosomes of D. willistoni and D. prosaltans.

Species	Chromosome-generations	Mutations observed	Frequency (percent)	Confidence limits
pseudoobscura	4082	40.78	0.999	0.733–1.340
persimilis	3820	68.12	1.783	1.403–2.264
willistoni	5715	51.12	0.894	0.678–1.179
prosaltans	6547	41.80	0.638	0.467–0.869

(according to DOBZHANSKY, SPASSKY and SPASSKY 1952) are summarized in table 2, together with the 95 percent confidence limits.

It can be seen that the mutation rates observed in *D. pseudoobscura* and *D. willistoni* are alike. That in *D. persimilis* is almost twice as high. The difference is statistically quite significant, since the confidence intervals do not overlap. *D. prosaltans* is the least mutable of the four species, although the confidence intervals overlap with those of *D. willistoni* and *D. pseudoobscura*. The mutation rate in the corresponding chromosome of *D. melanogaster* is unknown.

MUTATION RATES AND FREQUENCIES OF LETHALS AND SEMILETHALS IN NATURAL POPULATIONS

The mutation rates listed in table 2 may now be compared with the frequencies of the chromosomes in natural populations which are lethal or semilethal when homozygous. The data for the concealed lethals and semilethals are from DOBZHANSKY and SPASSKY (1953, 1954) and from PAVAN *et al.* (1951).

Species & locality	*Mutation rate (percent)*	*Accumulated lethals & semilethals (percent)*
persimilis (Yosemite)	1.783	25.5 ± 3.2
pseudoobscura (Yosemite)	0.999	33.0 ± 4.5
" (San Jacinto)	....	21.3 ± 1.8
willistoni (Brazil)	0.894	32.1 ± 1.4
prosaltans (Brazil)	0.638	9.5 ± 1.7

D. prosaltans has the lowest mutation rate among the four species, and also the lowest frequency of homozygous lethal chromosomes in its natural populations. The correlation between the mutation rate and the frequency of lethal chromosomes is, however, broken by *D. persimilis,* which is more mutable, and yet has fewer lethals, than *D. pseudoobscura* which lives in the same environment. A greater mutability evidently does not insure either a greater variability in populations, or, still less, a greater rate of evolutionary changes. This is because the persistence or elimination of a genetic variant in a large enough population depends on its effects on the fitness of heterozygotes much more than on that of homozygotes. Populations of very common and ecologically dominant species, such as *D. pseudoobscura* or *D. willistoni,* contain a great variety of linked gene combinations in their chromosomes. Any one kind of chromosome becomes homozygous only rarely in a large outbred species. As a consequence, a gene combination that is deleterious, or even lethal, when homozygous may nevertheless become a normal component of a population, provided that this gene combination is heterotic, i.e., increases the fitness of heterozygotes. Conversely, natural selection will fail to perpetuate a gene combination which is deleterious to heterozygotes (WALLACE and MADDEN 1953; CORDEIRO and DOBZHANSKY 1954, and other work). In a rare species the situation is different, because the adaptive effects of a gene combination in homozygotes as well as in heterozygotes are important from the standpoint of natural selection. As a consequence, *D. prosaltans* suffers from inbreeding less than the other species, and heterosis is also less important for the maintenance of its adaptedness to the environment.

MUTATION RATE AND THE TEMPERATURE OF THE HABITAT

In our previous publication (DOBZHANSKY, SPASSKY and SPASSKY 1952) we sought to explain the higher mutation rates observed in *D. willistoni* than in *D. prosaltans* in accord with the BERG-SCHMALHAUSEN hypothesis, according to which widespread, common, and ecologically versatile species should have higher mutation rates than rare and ecologically specialized forms. The data now at hand show that the phenomenon is more complex. *D. persimilis* is, if anything, an ecologically more specialized form than *D. pseudoobscura,* and yet it shows a mutation rate significantly higher than not only *D. pseudoobscura* but also *D. willistoni.* Indeed, the geographic distribution of *D. persimilis* is included into the much larger area inhabited by *D. pseudoobscura.* In the territory shared by both species, *D. persimilis* is characteristically an inhabitant of cooler, more humid, and seasonally less variable habitats. Although it often builds very large populations in suitable conditions, it acts

in the Sierra Nevada of California as a specialized subalpine and alpine form, without at the same time excluding *D. pseudoobscura* from these habitats.

An additional factor which may influence the mutation rates in a species or a race is the climate, and particularly the temperature, prevailing in the territory which it inhabits. This matter has been stated by MULLER (1942) as follows: "After organisms have lived for many generations at a high or very variable temperature, selection would have tended to counteract this environmental raising of the mutation rate by selecting genotypes which had an inherently lower rate than that which would otherwise be established. If this inference should prove justified—and it is entirely open to experimental testing . . . —then the differential influence of their natural temperatures on the mutation rates of two comparable species or geographical races living in different climates would not be nearly as great as the influence found on one and the same stock, when artificially separated into two groups and tested under these respective temperature conditions." Now, *D. persimilis* is characteristically an inhabitant of cooler climates than *D. pseudoobscura,* and this is most clearly apparent in the Yosemite region in which the strains used in our experiments were collected. In fact the experimental temperatures of 25°C are close to the upper limit of tolerance for *D. persimilis,* while *D. pseudoobscura* thrives at this temperature.

Examining from this standpoint the data of DOBZHANSKY, SPASSKY and SPASSKY (1952) we find the following fact which argues in favor of a relationship between climate and mutation rates. The mutation rates in the second chromosomes of *D. willistoni* of different geographic origin have been found to be as follows:

Geographic origin	*Mutation rate (percent)*	*Confidence limits*
Mogi, São Paulo	1.714	1.203–2.438
Pirassununga, São Paulo	0.762	0.472–1.231
Belem, Para	0.568	0.317–1.016

The Mogi locality is situated in the relatively cool upland of the State of São Paulo, in Southern Brazil; Pirassununga is one of the southernmost extensions of the savanna characteristic of Central Brazil; and Belem lies less than two degrees south of the Equator, to the south of the estuary of the Amazon. The mean temperatures are highest at Belem, lower at Pirassununga, and still lower at Mogi.

No data on the possible variations in the mutation rates in different geographic races of *D. pseudoobscura* or *D. persimilis* are available. However, DOBZHANSKY and WRIGHT (1941) have examined the mutation rates producing lethals and semilethals in the third chromosomes of *D. pseudoobscura* from Mount San Jacinto, in southern California, which is a region with a warmer climate than that in the Sierra Nevada. The frequency of mutation was found to be 0.307 percent per generation, with the 95 percent confidence limit 0.240–0.393 percent. This is only one-third of the mutation rate in the

second chromosomes from the Yosemite region (table 2). Now, the third chromosome in *D. pseudoobscura* is cytologically somewhat shorter than the second, and it contains fewer lethals in natural populations (25.0 ± 4.0 percent compared to 33.0 ± 4.5 percent, see table 4, DOBZHANSKY and SPASSKY 1953). The difference is, however, nowhere near as great as needed to account for the much smaller mutation rate found in the San Jacinto population. We now believe that both the ecological status of a species and the climate of its distribution area are involved in the determination of the mutation rates in this species.

SUMMARY

The rates of spontaneous mutations giving rise to recessive lethals and semilethals in the second chromosomes of *Drosophila pseudoobscura* and *Drosophila persimilis* have been studied. Strains of both species derived from the wild progenitors collected in the same localities in the Yosemite Park region of the Sierra Nevada of California have been used.

The mutation rates are significantly higher in *D. persimilis* than in *D. pseudoobscura* (table 2). This fact cannot be accounted for on the basis of the BERG-SCHMALHAUSEN hypothesis, which postulates that mutation rates should be, other things being equal, higher in widespread and ecologically versatile than in ecologically more specialized forms. It fits, however, the hypothesis of Muller, according to which the inhabitants of warmer climates should have, other things being equal, lower mutation rates at similar temperatures than do the inhabitants of cooler territories.

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LITERATURE CITED

CORDEIRO, A. R., and TH. DOBZHANSKY, 1954 Combining ability of certain chromosomes in *Drosophila willistoni* and invalidation of the "Wild-Type" concept. Am. Nat. (in press).

DOBZHANSKY, TH., and C. EPLING, 1944 Contributions to the genetics, taxonomy, and ecology of *Drosophila pseudoobscura* and its relatives. Carnegie Inst. Wash. Publ. **554**: 1–183.

DOBZHANSKY, TH., and B. SPASSKY, 1953 Genetics of natural populations. XXI. Genetics **38**: 471–484.

1954 Genetics of natural populations. XXII. Genetics **39**: 472–487.

DOBZHANSKY, TH., B. SPASSKY and N. SPASSKY, 1952 A comparative study of mutation rates in two ecologically diverse species of Drosophila. Genetics **37**: 650–664.

DOBZHANSKY, TH., and S. WRIGHT, 1941 Genetics of natural populations. V. Genetics **36**: 23–51.

DUBININ, N. P., 1946 On lethal mutations in natural populations. Genetics **31**: 21–38.

IVES, P. T., 1950 The importance of mutation rate genes in evolution. Evolution **4**: 236–252.

LEVINE, R. P., and P. T. IVES, 1953 Mutation rates and lethal gene frequencies in populations of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **39**: 817–823.

MULLER, H. J., 1942 Isolating mechanisms, evolution, and temperature. Biological Symposia **6**: 71–125.

PLOUGH, H. H., 1942 Temperature and spontaneous mutation. Biological Symposia **6**: 9–20.

SPASSKY, B., and TH. DOBZHANSKY, 1950 Comparative genetics of *Drosophila willistoni*. Heredity **4**: 201–215.

WALLACE, B., and C. V. MADDEN, 1953 The frequencies of sub- and supervitals in experimental populations of *Drosophila melanogaster*. Genetics **38**: 456–470.

NATURAL MUTATIONS IN INBRED LINES OF MAIZE AND THEIR HETEROTIC EFFECT. I. COMPARISON OF PARENT, MUTANT AND THEIR F_1 HYBRID IN A HIGHLY INBRED BACKGROUND[1]

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IT has been known for over 50 years that inbreeding reduces vigor of the offspring and crossing inbreds restores normal vigor. Frequently the F_1 exceeded the better of the two original parents. Utilization of this increase in vigor was the basis for production of varietal hybrids in corn, and later the development of corn hybrids from inbred lines. Principles used in hybrid corn spread to other crops such as sugar beets, melons and onions, and to swine, poultry and other animals. In view of the economic importance of heterosis, it is surprising that so few critical experiments have been conducted to establish the genetic mechanism involved. It appears probable that further advances in commercial utilization of heterosis may well depend upon further clarification of the types of gene action involved.

SHULL (1914) coined the term "heterosis" to replace the lengthy expression "stimulus of heterozygosity" and also to provide a term which carried no implication as to genetic mechanism involved. Even before the term heterosis was proposed, studies had been reported which were designed to explain the phenomena. The explanations proposed fell into two rather distinct categories. Without attempting to follow the changes in terminology or assumptions as to mechanisms involved, two opposing theories still persist and are commonly designated by the simple terms dominance and overdominance. The former assumes that the combination of dominant or partially dominant favorable growth factors bring about hybrid vigor, while the latter rests on the assumption that the heterozygous state *per se* results in some kind of physiological stimulus expressed as hybrid vigor. In simple genetical symbols the dominance hypothesis assumes that $AA \geqslant Aa$ and the overdominance hypothesis that $Aa > AA$ where A is the dominant, and a the recessive gene. The smallest unit in which overdominance can be studied is the single gene. A gene would show overdominance or a locus would be heterotic if the heterozygote exceeded either homozygote.

Long time inbred lines of maize are assumed to be highly homozygous. If a mutation in such a line occurred at a single locus, it would be possible to

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produce and compare the three possible genotypes *AA*, *Aa* and *aa* in an identical genetic background. Superiority of the *Aa* plants could be attributed to heterotic effects of the single gene under consideration or to other sources of heterozygosity not under direct control. It seems rather important to investigate whether the basic assumption of a one-gene difference holds or whether other heterozygous loci are involved. A crude way to accomplish this would be to isolate one or both of the homozygous genotypes by selfing the heterozygote and to compare the recovered homozygous line with the corresponding parental line, which had not gone through a cross generation since the time the mutation was discovered and isolated.

Numerous older reports in which the heterozygote was superior in some attributes to either homozygote were brought to attention by advocates of the overdominance hypothesis. In the past 30 years, a rather large number of papers have been published on overdominance at a single locus. In most of these studies, the genes under study were not in a homozygous background as e.g. in Drosophila and other animals. In plants, QUINBY and KARPER (1946) compared lines of sorghum which were presumed to differ only in a number of genes for maturity. It was found that plants heterozygous for one of the maturity genes were larger than homozygous plants and this difference was ascribed to one locus. In a previous paper by the same authors in 1945, where the derivation of the lines used in the heterosis study was described, it is apparent that the lines were not isogenic but were segregates obtained after crossing different strains of milo.

HAGBERG (1953) presented an analysis of several cases of monofactorial heterosis in a series of so-called "erectoides" (dense ear) mutants in barley. The mutations were X-ray induced. Overdominance on a single locus was found in two-gene combinations for total plant weight, yield of grain per plant and tillering, one of them also in number of florets per plant.

Natural mutations affecting chlorophyll synthesis, which had occurred in the pure line Golden Barley, were studied by GUSTAFSSON (1946, 1947). The heterozygotes were reported to be superior in one or more of the characters measured, although no data were presented to indicate the significance of the results. When two loci were heterozygous, the heterotic effect seemed to be cumulative and differences in many attributes were significantly in favor of the heterozygous class.

SINGLETON (1943) crossed a semi-dwarf mutant with the inbred line of corn in which the mutation had occurred as well as to an unrelated inbred line. In the former cross the genotype heterozygous for the semi-dwarf locus significantly exceeded the parent line in rate of growth and ear weight. In test crosses to an unrelated source, the cross involving the recessive semi-dwarf gene gave a significant increase over the cross carrying both dominant alleles.

A number of heritable morphological alterations in corn, presumably mutations, were intercrossed with their respective parent lines and the F_1 then compared with both homozygous classes (JONES 1945). All hybrids were

significantly superior in one or more attributes. In a later article, JONES (1952) stated, ". . . but the results to date indicate that the differences involved are not single genes." No data have been published, although the above remark indicates that the assumption of a single gene difference was not correct.

MATERIALS AND METHODS

In long time inbred lines of corn occasional plants may be found which differ sufficiently from their full sibs in one or more morphological characteristics as to be easily recognizable. Several such off-type plants, thought to be the result of single gene mutations, have been found and maintained (table 1). A total of 14 pairs of lines, namely the original and its corresponding mutant line, were grown in 1951 in adjacent single row plots to make crosses between the pair as well as to sib some plants within each row. The mutant line always served as a male parent. Desired amounts of seed of each of the three possible genotypes *AA*, *Aa* and *aa* were obtained for only eight of the 14 pairs of lines, namely narrow leaf, sugary 205, sugary GG824, brachytic, green stripe, male sterile 317, dwarf SW1 and dwarf 187-2. The three genotypes of the eight mutants were grown in 1952 in eight separate randomized block designs with from 7 to 16 replications. These tests will be called "mutant tests." A plot consisted of a single 10-plant row, with plants spaced approximately 13 inches apart in the row. The following measurements were taken on an individual plant basis: plant height in centimeters, total weight of shelled seed per plant in grams, total number of kernels per

TABLE 1

Designation, symbol, nature, origin and history of mutants, and year of test.

Name of mutant	Genetic symbol	Nature of mutation	Found by or obtained from	Generations of selfing or sibbing		Test grown in
				Prior to mutation	After mutation	
Narrow leaf	*nl*	Recessive	Sprague	10	4	1952
Sugary	*su 205*	Recessive	Sprague	20	5	1952
Male sterile	*ms 317*	Recessive	Sprague	20	5	1952
Brachytic	*br*	Recessive	Sprague	20	2	1952
Green stripe	*gs*	Recessive	Sprague	5	3	1952
Sugary	*su GG824*	Recessive	Sprague	10	5	1952
Dwarf	*d SW1*	Recessive	Pioneer	5	3	1952
Dwarf	*d 187-2*	Recessive	Pioneer	5	3	1952
Crinkled	*cr*	Recessive	Sprague	5	4	1953
Small seed	*sm M14*	Recessive	Sprague	10	3	1953
Grass like	*gr*	Recessive	Rubis	20	3	1953
Red pericarp	*P*	Dominant	Sprague	10	10	1953
Male sterile	*ms 38-11*	Recessive	Sprague	20	4	1953
Brown midrib	*bm*	Recessive	Sprague	4	1	1953
Small seed	*sm 1373*	Recessive	Sprague	5	1	1953
Dwarf	*d 187-2*	Recessive	Pioneer	5	3	1953

plant, kernel row number and ear length in millimeters. Ear diameter in millimeters, leaf width in millimeters, and number of tassel branches were recorded only in the narrow leaf mutant test.

Seed for seven more mutant tests was produced in 1952 and in the greenhouse in 1952/1953. They were crinkled, small seed M14, grasslike, red pericarp, male sterile 38-11, brown midrib and small seed 1373. Randomized block designs with the same plot size were used, except that the *aa*-genotype was omitted, because from the analyses of the previous year it was learned that the homozygous recessives were of no particular interest in this type of analysis. The mutant test of dwarf 187-2 was repeated in 1953, except for the omission of the homozygous recessive sibs. Characters measured in 1953 were the same as in 1952, except for the substitution of 100-kernel weight for kernel number. The mutant tests for brown midrib and red pericarp were inconclusive because in each case one of the parents, although presumed to be homozygous, was found to be heterozygous.

In 1952 seed was collected from heterozygous plants which were either selfed or backcrossed to the homozygous recessive stock. Their progenies were grown in an attempt to recover the homozygous recessive mutant type. Selfs were obtained for the lines narrow leaf, sugary 205, sugary GG824, and green stripe, and backcrosses for brachytic and dwarf SW1. For these mutants the double recessive segregates were phenotypically separable and only on them were data recorded. Such recovered or extracted homozygous recessives could be compared with or tested against the original recessive stock. These experiments are called " recovery tests " and had only two entries, namely the progeny of the selfed or backcrossed ear and a mechanical mixture of the dominant parent line and the original recessive mutant. In both entries data were collected only on the homozygous recessive mutants. The purpose of the mixture was to provide the smallest degree of bias in border effects by simulating the segregation in the entries planted with selfed or backcrossed seed. Recovery tests were laid out as randomized blocks and handled in the same manner as the mutant tests.

Within each mutant and recovery test an analysis of variance was performed for each attribute measured. Only data from the homozygous dominant and the heterozygous plants were used in the mutant tests in 1952 because the homozygous recessive class was constantly and distinctly inferior. Furthermore, the most interesting comparison is represented by the *AA* class vs. the *Aa* class. All mutant tests in 1953 had only two entries, namely, the *AA* vs. the *Aa* line. To improve classification or separation in both the mutant and the recovery tests three attributes were combined to form a discriminant function. The technique was developed by FISHER (1936) and demonstrated by COX and MARTIN (1937) and others. An analysis of variance was calculated for each attribute separately and for the discriminant. A t-test of the discriminant also was calculated.

TABLE 2

Means of the three genotypes AA, Aa and aa for each of the attributes taken on eight mutants in 1952.

Mutant	Geno-type	Height in centimeters	Number of seeds	Total kernel weight in grams	Kernel row number	Ear length in millimeters	Leaf width in millimeters	Number of tassel branches	Ear diameter in millimeters
	AA	156.18	795.51	141.20	12.02	151.44	81.53	13.79	40.59
nl	*Aa*	155.78	736.36	133.55	11.90	148.75	81.30	12.72	39.90
	aa	155.40	319.30	52.00	10.30	130.50	44.40	7.60	33.70
	AA	217.73	230.53	63.98	14.94	147.02			
su 225	*Aa*	218.93	286.06	81.75	16.59	159.10			
	aa	212.00	154.00	49.00	13.00	143.00			
	AA	230.13	553.78	103.20	14.09	199.24			
ms 317	*Aa*	226.16	518.18	97.18	14.27	200.36			
	aa	....	....	....	....	....			
	AA	134.82	460.19	107.71	18.58	173.88			
br	*Aa*	138.39	465.19	109.45	18.37	166.24			
	aa	84.80	341.50	70.70	18.30	156.30			
	AA	152.88	329.44	91.63	16.58	164.59			
gs	*Aa*	148.67	426.20	120.68	17.26	185.77			
	aa	139.20	236.20	47.30	15.20	147.20			
	AA	170.60	96.46	18.56	14.24	96.84			
su GG824	*Aa*	172.36	92.93	20.18	14.48	109.18			
	aa	182.60	76.40	10.60	14.50	96.80			
	AA	188.58	701.65	168.23	15.43	214.06			
d SW1	*Aa*	182.04	917.34	237.43	15.31	232.73			
	aa	92.30	426.90	81.40	15.20	174.90			
	AA	179.05	530.98	116.27	13.70	190.79			
d 187-2	*Aa*	192.46	724.61	185.82	15.72	221.61			
	aa	83.70	138.30	30.30	10.60	107.00			

EXPERIMENTAL RESULTS

Mutant tests

The means of the three genotypes for each of the eight mutant tests grown in 1952 are summarized in table 2. The homozygous recessive class was the least vigorous in all but two of the 38 cases for which measurements on recessives were available. The recessives were not included in the analyses of variance (table 4). The means of the mutant tests grown in 1953 are shown in table 3. The F-values for tests grown in 1952 and 1953 are presented in table 4.

In the narrow leaf mutant, comparing the dominant vs. the heterozygous class, all differences were in favor of the double dominant genotype, but only for kernel row number was the difference significant at the 5 percent level.

TABLE 3

Means of the two genotypes AA and Aa for each of the characters measured on five mutants in 1953.

Mutant	Genotype	Height in centimeters	Kernel row number	Ear length in millimeters	100-kernel weight in grams	Total kernel weight in grams
cr	*AA*	196.15	15.76	191.36	25.78	86.26
	Aa	222.38	17.47	221.29	27.46	139.17
sm M14	*AA*	180.76	16.85	210.28	20.83	124.82
	Aa	178.12	16.36	216.38	21.58	128.14
gr	*AA*	201.55	15.81	175.15	31.62	125.35
	Aa	190.07	15.11	175.79	30.40	113.62
sm 1373	*AA*	172.25	13.37	188.53	26.93	126.18
	Aa	173.11	13.60	184.73	26.68	116.95
d 187-2	*AA*	184.38	13.61	194.78	24.49	68.64
	Aa	207.85	14.40	216.74	26.07	132.42

In the sugary 205 test the heterozygous allele combination was superior to the homozygous dominant genotype and all differences except for plant height were highly significant. Plant height was the only attribute for which significant differences were found in the male sterile 317 and the brachytic line. In the former the difference was in favor of the dominant class; in the latter the heterozygotes were superior. In the green stripe test all analyses gave a highly significant F-value and, with the exception of plant height, the heterozygotes were superior to the dominant parent. The only significant difference obtained in the sugary GG824 line was for ear length, the heterozygote having longer ears. In both dwarf mutants differences in all but two of the attributes measured were highly significant; the heterozygotes always exhibited the greater mean. The test for the dwarf 187-2 mutant, which was repeated in 1953, showed the same trends as in 1952. The heterozygotes for crinkled exceeded significantly the homozygotes in all characters. In the small seed M14 all but one of the differences were non-significant. The same held

TABLE 4

The variance ratios obtained in the analyses of variance of the heterozygous vs. the homozygous dominant genotype for all attributes measured on all mutants in 1952 or 1953.

Mutant	Height	Number of seeds	Total kernel weight	Kernel row number	Ear length	100-kernel weight	Leaf width	Number of tassel branches	Ear diameter
nl	.04	1.11	.42	5.00*	4.10	....	.13	4.39	3.38
su 225	.56	10.73**	18.81**	66.00**	43.22**				
ms 317	5.77*	.54	.68	3.09	.21				
br	12.14**	.25	.19	.62	.56				
gs	13.75**	34.27**	47.03**	6.41*	65.27**				
su GG824	.47	.31	.66	.86	93.83**				
d SW1	1.53	19.99**	29.86**	.73	21.10**				
d 187-2	75.51**	72.18**	371.31**	115.37**	253.30**				
cr	136.26**	....	25.01**	115.00**	41.41**	8.21*			
sm M14	4.53	....	.68	6.03*	2.31	.79			
gr	32.21**	....	3.28	2.80	.03	5.71*			
sm 1373	.26	....	12.10**	1.10	2.25	.18			
d 187-2	205.93**	....	144.67**	24.38**	94.42**	17.78**			

The homozygous recessive entry was omitted in all analyses.
*Exceeds the 5 percent level of significance.
**Exceeds the 1 percent level of significance.

for the second small seed line. The homozygous dominant plants in the grass-like mutant test exceeded the heterozygotes significantly in plant height and 100-kernel weight. In the test of the male sterile 38-11 no stand was obtained.

For every one of the 12 mutant tests a discriminant function consisting of three attributes was calculated. Then each function's discriminatory value was evaluated by an F-test and a t-test, as shown in table 5. The relative value of each of the three attributes is represented by the magnitude of the coefficient attached to each selected character. For both dwarf mutants the same three characters were chosen, namely, plant height, total kernel weight and ear length. In the dwarf SW1 experiment, plant height was not significant, but nevertheless it had the highest discrimination coefficient demonstrating that neither the F- nor t-test provided an entirely reliable criterion in selecting the attributes, because possible correlations among the attributes are disregarded. In case of the dwarf 187-2 test, which was repeated in 1953, the same three attributes were chosen as for the 1952 test. The correlation coefficients changed considerably from year to year. The rank of the discriminatory value of the three attributes was unaltered but the magnitudes of the individual coefficients were quite dissimilar in the two years.

In general in every mutant test a highly significant F-value was obtained for the discriminant, and in all cases the misclassification frequency of the discriminant was lowered as compared to any single attribute.

Recovery tests

An attempt was made to secure selfed or backcrossed seed only for the mutant tests grown in 1952. No seed for the male sterile 317 was attempted. The backcross seed of the dwarf 187-2 heterozygote was lost accidentally. Seeds homozygous for sugary 205 and sugary GG824 germinated so poorly that both recovery tests were abandoned. Viability of plants homozygous for brachytic was reduced to such an extent that the final stand was too poor to make an analysis worthwhile.

In both the plots with segregating progenies and with mixtures, plant counts were undertaken at pollination and at harvesting time. Expected ratios were in many cases quite distorted, which probably was due to differential viability of the different genotypes. This differential viability was not always consistent in the two entries of a test.

Means and F-values are summarized in table 6. In the recovery tests for narrow leaf, green stripe and dwarf SW1 a discriminant was obtained and an F- and t-test made (table 7). The three attributes selected for the combined analysis are the same as for the discriminant of the mutant test with the exception of the narrow leaf recovery test where ear length was substituted for leaf width. The difference between the two sources of recessives for all three mutations were highly significant when the discriminant was used as means of separation. Misclassification frequencies ranged from < 15 to < 30 percent.

TABLE 5

Linear discriminants obtained, their significance and frequency of misclassification resulting in all mutant tests.

Mutant	Discriminant[a]	F-value	Error of misclassification using									
			Z	H	N	W	R	L	S	Lw	T	E
nl	.011,975T + .024,544E + .066,501L	3.38*	.30					.40			.40	.40
su 225	.001,258W − .298,451R − .025,156L	57.79**	.005			.25	.10	.15				
ms 317	.018,542H + .000,206N − .175,340R	6.83**	.20	.35	.45		.45					
br	−.036,130H + .129,945R + .002,471L	13.08**	.15	.30			.45	.45				
gs	.015,417H + .012,000W − .014,969R	31.63**	.05	.30		.15	.35					
su GG824	−.228,145W + 1.120,761R − .729,303L	106.17**	.0005			.45	.45	.025				
d SW1	.005,159H − .003,927W − .002,025L	25.63**	.10	.45		.20		.25				
d 187-2	−.059,918H − .037,687W − .056,983L	386.84**	.0005	.01		.005		.01				
cr	−.045,860H − .563,908R − .011,671L	187.90**	.0005	.05			.05	.15				
sm M14	.013,618H + .091,928R − .004,106L	7.92**	.20	.40			.35	.40				
gr	.033,532R + .094,600S + .003,433W	5.85**	.25			.35	.35		.30			
sm 1373	−.048,807R − .003,240L + .013,087W	8.94**	.20			.30	.45	.40				
d 187-2	−.061,740H − .004,807W − .023,267L	169.24**	.0005	.025		.025		.10				

[a]Symbols represent Z discriminant function; H plant height; N kernel number; W total kernel weight; R kernel row number; L ear length; S 100-kernel weight; Lw leaf width; T number of tassel branches; E ear diameter

TABLE 6

Means and F-values for all characters measured on four mutants in the recovery tests.

Mutant	Source of recessive and F-value	Plant height in centimeters	Kernel row number	Ear length in millimeters	100-kernel weight in grams	Total kernel weight in grams	Leaf width in millimeters	Number of tassel branches
	Original	155.60	9.97	138.36	18.20	66.65	39.93	7.09
nl	Extracted	154.57	10.35	129.41	17.83	63.23	43.16	8.20
	F-value	.10	2.63	1.91	.71	.20	7.57*	7.48*
	Original	80.80	17.33	166.13	20.13	66.33		
br	Extracted	80.40	18.60	164.60	21.00	94.80		
	F-value[a]	....	....	....	....	....		
	Original	121.76	14.61	139.78	24.18	31.80		
gs	Extracted	133.02	15.62	130.54	21.74	45.47		
	F-value	14.40**	2.98	1.56	1.31	6.87*		
	Original	107.38	15.44	197.58	21.52	101.65		
d SW1	Extracted	101.48	15.55	186.39	20.59	87.37		
	F-value	4.47*	0.00	8.02**	2.36	3.51		

[a]Not enough recessives were obtained to make an Analysis of Variance.
*Exceeds 5 percent level of probability.
**Exceeds 1 percent level of probability.

TABLE 7

Linear discriminants obtained, their significance and frequency of misclassification resulting in all recovery tests.

Mutant	Discriminant[a]	F-value	Errors of misclassification using						
			Z	H	W	R	L	Lw	T
nl	.016,787Lw + .048,057T − .000,518L	10.77**	.20	....	....	....	.45	.35	.35
gs	−.019,744H − .004,947W − .006,410R	9.78**	.15	.25	.30	.40	....		
d SW1	.000,883H + .001,612L − .000,118W	5.63**	.30	.40	.45	....	.40		

[a]For symbols see table 5.

DISCUSSION

Mutant tests

For breeding purposes a practical solution to the heterosis problem would consist in determining the relative importance of dominance and overdominance. SPRAGUE and MILLER (1950) have devised a scheme which may permit the evaluation of the relative importance of the two kinds of gene action *en masse*. Limited data have been presented (SPRAGUE and MILLER 1953) and are in agreement with the dominance hypothesis. An answer to the relative importance, however, would not provide critical evidence to the basic issue and would not permit phrasing a basic law for the individual and universal case. One of the major difficulties of the past has been the fact that many experiments reported in the literature may be explained equally satisfactorily by either the dominance or the overdominance hypothesis. It is a common belief among breeders and geneticists that both types of gene action are involved.

The phenotypic effect of a gene is a result of some primary component, such as catalytic or enzymatic action. Only in rare instances, if ever, are we able to measure these primary components. Ordinarily, genes are characterized only by their end effects. The latter is the case in this study because here a number of attributes were recorded on genotypes presumably differing by one or two alleles. The concept of dominance in such cases frequently needs to be restated in terms of each partial effect separately rather than considered " en masse," because the homozygous allele pair may have positive and negative partial effects upon the phenotype although the primary effect may be either minus or plus. Upon observing the sum of secondary effects, a combination of the two alleles appears to operate in the sense of single locus overdominance. The same holds for cases of close linkage in the repulsion phase.

The above demonstrates the difficulties involved in what may be called a phenotypic approach, and it emphasizes the necessity and possibilities of biochemical methods. But for biochemical studies to be effective on single locus heterosis requires genetic stocks which differ in only one allele. Such material at the present time cannot be secured. Fairly large numbers of single gene mutations or point mutations in long time inbred lines of maize are the closest approximation available at the present time.

The concept of point mutation is a working hypothesis. No cytological or genetical tools are available to classify positively a heritable change as a point mutation. Gross changes in the chromosomal make-up are detectable by cytological examination, but small unexposable inversions, duplications, deficiencies and rare cross-overs may give mutation-like effects. These difficulties are especially important in heterosis studies where X-ray induced mutations are used. When X-ray treatments are involved it appears probable that changes without phenotypically visible effects are likely to have occurred.

Another method to obtain lines which differ by one allele consists in con-

tinuous back-crossing of a mutant to a homozygous recessive stock. The expected end result is a second homozygous stock identical to the recurrent parent in all but the mutant locus. A second way of obtaining such lines is to cross two lines, select in each generation a progeny plant heterozygous for the locus studied while the other loci approach homozygosity by continued selfing. After an adequate number of selfed generations, contrasting lines are selected. The difficulty inherent in the production of isogenic lines by backcrossing or selfing is the unlikeliness of replacement of genes located close to the locus concerned. Hence, resulting lines may be largely isogenic but may differ with respect to short chromosomal segments on which the mutants are located. It seems reasonable that the best approximation to ideal isogenic conditions are cases where mutations occurred in long time inbred lines, clones, identical twins, etc. Neighboring chromosomal regions probably would remain constant.

Maize is a rather favorable subject for this kind of study. It is genetically and cytologically more thoroughly investigated than any other plant species and it can be easily selfed and crossed. Drawbacks are that we depend upon natural mutations of a conspicuous form which occur with low frequencies. The mutant stock as well as parent lines must be grown from time to time to maintain viable seed. Each such selfed or sibbed generation exposes the gene complex to new mutations. The approximate number of selfed or sibbed generations for the mutants and their original stocks is shown in table 1. Complete homozygosity of even long-time inbred lines cannot be claimed, because of the possibility of relic heterozygosity (heterozygosity not eliminated by inbreeding). There is no reliable information on the importance of relic heterozygosity or newly occurring mutations in inbred lines of maize. In spite of all these obstacles the use of natural mutations in inbred lines of corn appears to be one of the more critical techniques available for the investigation of single locus heterosis.

It is recognized that the 12 mutations in this study with which tests were completed are neither a random nor an adequate sample of all loci. They are all recessive mutations which probably mutate with higher than average frequency and have above average phenotypic appearance. In spite of these limitations, this sample is the largest collection of such mutations in maize specifically tested for their heterotic effect in supposedly isogenic lines. JONES (1944, 1945) described six heritable changes in corn and conducted tests similar to the one reported in this paper. A number of irregularities in segregating progenies and instability of lines were mentioned in the 1945 articles and he later (1952) stated that the differences were not of a single gene nature.

If heterozygosity *per se* is advantageous, then all heterozygous loci should exhibit overdominance, which was true for the six mutants reported by Jones. A more realistic assumption would be that an overdominant type of gene action exists for only a limited number of loci. In that event one would expect to find heterotic and non-heterotic loci in a random sample. The 12 mutants in this study seem to bear out this assumption. The loci sugary 205,

green stripe, dwarf SW1, dwarf 187-2 and crinkled definitely appear to be examples of heterotic allele combinations in that all or almost all variables were significantly higher in the heterozygotes (tables 2, 3 and 4). The contrasting group of alleles was comprised of narrow leaf, male sterile 317, grass-like and small seed 1373. Here none of the differences was in favor of the heterozygotes and if significant differences existed, the dominant parent was the better. The third or intermediate group was indefinite. Differences were small, varied from one attribute to the other and were rarely significant. Cases of this type were brachytic, sugary GG824 and small seed M14.

The above classification of the 12 mutants is based on the analysis presented in table 4, without utilizing the discriminant function approach. Separation of the two genotypic classes for all mutants can be improved by the use of the discriminants as seen from the lowered errors of misclassification (table 5). The validity of the discriminant function approach may be questioned on grounds that the attributes in many cases, were selected according to their F-values and not at random. However, under the assumption of a single gene difference it must be assumed that the magnitude of the means of the contrasting genotypes was due to this one locus.

Recovery tests

As discussed in the foregoing section, relic heterozygosity and new mutations constitute a source of variability which could invalidate results obtained in the mutant tests. In none of the previous reports in the literature on single locus heterosis had an attempt been made to investigate the importance of these factors. JONES (1952) stated without presenting any data, that in the material reported in 1945 ". . . some other changes must have been associated with the variable condition." Usually the authors justified their assumption of a single gene difference by establishing a monohybrid ratio.

In view of the two conceivable sources of variability in supposedly isogenic lines, and the lack of critical evidence in the literature in support of a single locus difference, the recovery tests were thought to be essential. Unfortunately, for reasons mentioned in Materials and Methods, only three of these experiments were analyzable, namely narrow leaf, green stripe and the dwarf SW1 mutants. The two latter loci were previously classified as possible heterotic loci, while the narrow leaf alleles showed no such gene action. In spite of the small number of recovery tests the results were surprisingly uniform in that all experiments gave highly significant F-values for the variance ratio in the analyses of variance of the discriminant functions. In the case of the two heterotic mutants this would indicate that the assumption of a single locus difference was not correct and that other sources of variability were present in the supposedly isogenic line pairs. The separation of the two sources of recessives for the green stripe and dwarf SW1 mutants was effective by the same combination of attributes as was used the previous year in the mutant tests. The significant F-value for the narrow leaf discriminant of the recovery test merely points out that here also the two members of the

isogenic line must have differed by more than the narrow leaf locus. The source of this additional variability may be new mutations arising during the generations of selfing or sibbing following the discovery of the mutant or it may be relic heterozygosity present in the inbred line at the time the mutation occurred. In corn it would be rather difficult to eliminate these two possibilities, unless an intensive search is undertaken for mutations in one-year-old inbred lines derived by the haploid method. If the indication of unsuspected variance in isogenic lines, as disclosed by this study, are of general nature, then practically all of the most critical experiments of the past are rendered invalid.

The disproval of the assumption underlying the mutant tests does not exclude that these loci may have had overdominance-like effects. But it does point out that what other investigators have labeled as single gene heterosis may likely be due to a multigenic diversity. Further, no conclusions can be drawn in respect to the degree of importance of cumulative small heterotic effects nor that such effects may not be of importance. On the other hand, it is felt that present evidence on types of gene action and interaction such as dominance and epistasis can account for the heterosis phenomenon in a satisfactory manner.

SUMMARY

1. Dominance and/or overdominance are currently and commonly the two hypotheses advanced for explaining the heterosis phenomenon. Superiority of the heterozygous genotype over both homozygous classes for a single locus with a homozygous genetic background would be evidence for one type of overdominance.

2. Seed of the three possible genotypes *AA, Aa* and *aa* for each of 15 mutants, which had occurred in long time inbred lines, was produced and tested in randomized block experiments. The tests in 1952 included all three genotypes, while in 1953 the homozygous recessive class was omitted. Measurements on an individual plant basis were recorded and a total of 12 mutants analyzed.

3. Selfing or backcrossing plants heterozygous for the mutation resulted in recovered homozygous recessives which were compared in randomized blocks with the original recessives. It was possible to analyze recovery tests for only three mutants (narrow leaf, green stripe and dwarf SW1).

4. A separate analysis of variance was calculated for each attribute measured in each of the mutant and recovery tests. For each mutant and recovery test three attributes were chosen and a discriminant function calculated. Analyses of variance for the discriminant were presented. T-tests of the discriminant and the individual attributes permitted a comparison of misclassification frequencies.

5. According to the results obtained in the mutant tests, the 12 mutants were classified into three groups: 1) mutants where the heterozygous genotype was distinctly superior in all or many of the attributes measured, 2) mutants

where no heterotic behavior was apparent, 3) mutants where a classification was intermediate or erratic.

6. The variance ratio of the discriminant was highly significant in all three recovery tests indicating that the two sources of plants homozygous for the recessive mutant were not identical with respect to loci other than the mutant genes. These results preclude the acceptance of heterotic single loci effects as obtained in the mutant tests. Similarly, previously published experiments by a number of authors might have revealed such discrepancies if recovery tests had been conducted.

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LITERATURE CITED

COX, G. M., and W. P. MARTIN, 1937 Use of a discriminant function for differentiating soils with different Azotobacter populations. Iowa State College J. of Science **11**: 323–332.

FISHER, R. A., 1936 The use of multiple measurements in taxonomic problems. Ann. Eugenics **7**: 179–188.

GUSTAFSSON, ÅKE, 1946 The effect of heterozygosity on variability and vigour. Hereditas **32**: 263–286.

1947 The advantageous effect of deleterious mutations. Hereditas **33**: 573–575.

HAGBERG, A., 1953 Heterozygosity in erectoides mutations in barley. Hereditas **39**: 161–178.

JONES, D. F., 1944 Equilibrium in genetic materials. Proc. Nat. Acad. Sci. U.S.A. **30**: 82–87.

1945 Heterosis resulting from degenerative changes. Genetics **30**: 527–542.

1952 Plasmagenes and chromogenes in heterosis. In Gowen, J. W., ed., Heterosis, pp. 224–235. Ames, Iowa, The Iowa State College Press.

QUINBY, J. R., and R. E. KARPER, 1945 The inheritance of three genes that influence time of floral initiation and maturity date in milo. J. Amer. Soc. Agron. **37**: 916–936.

1946 Heterosis in sorghum resulting from the heterozygous condition of a single gene that affects duration of growth. Amer. J. Bot. **33**: 716–721.

SHULL, G. H., 1914 Duplicate genes for capsule form in *Bursa bursa-pastoris*. Zeit. ind. Abst. Ver. **12**: 97–149.

SINGLETON, W. R., 1943a Hybrid vigor in the intra-inbred cross P39 × C30 in maize. Record of Genetics Soc. of Amer. **12**: 52–53.

1943b Breeding behavior of C30 diminutive P39 mutant whose hybrids show increased vigor. (Abstract) Genetics **28**: 89.

SPRAGUE, G. F., and P. A. MILLER, 1950 A suggestion for evaluating current concepts of the genetic mechanism of heterosis in corn. J. Amer. Soc. Agron. **42**: 161–162.

1952 Evidence on types of gene action involved in yield of corn. (Abstract) Amer. Soc. Agron. Meetings 1952: **19**.

A GENETIC ANALYSIS OF REVERSED ACROCENTRIC COMPOUND X CHROMOSOMES IN DROSOPHILA MELANOGASTER [1]

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TWO X chromosomes in Drosophila can be joined together to produce a single chromosome which is called a compound X chromosome, the best known example of which is the attached-X chromosome. There are five other types of compound X chromosomes possible, however, since the two component chromosomes may either be in tandem or reversed order with respect to one another; the centromere may either be between them or at one end; and the compound may either be closed to form a ring or not. All six of the possible compound X chromosomes have now been synthesized (NOVITSKI 1954). Of these six, however, only two, the attached-X and the tandem metacentric (equals tandem attached) X, have been analyzed to any great extent.

X-ray induced reversed acrocentric compound X chromosomes, known also as double X chromosomes, were first described by MULLER (1943, 1944) and by VALENCIA, MULLER and VALENCIA (1949), who pointed out the consequences of crossing over in this type of compound. The reversed acrocentric compound pairs with itself in the same way as a simple attached-X chromosome, but differs from the attached-X in that the centromere is located at the free end of one of the components. The chromosomes produced by these workers were limited in their usefulness for purposes of analysis, however, since one of the component chromosomes carried an inversion, dl-49. The detection of spontaneous reversed acrocentric compound X chromosomes, and crossover data from these, have been reported by NOVITSKI (1954).

The present work represents an analysis of the origin and meiotic behavior of spontaneous reversed acrocentric compound X chromosomes. It will be shown below that a large proportion of the newly arisen compounds are of such a nature as to exclude their origin by a simple event; other possibilities will be discussed. In addition, it will be shown that crossing over within the compounds is markedly different from that which would be predicted on the basis of studies of other compounds and unattached X chromosomes in that there appears to be a pronounced deficiency of single, as compared with double, exchanges.

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ORIGIN OF REVERSED ACROCENTRICS

Production of reversed acrocentrics

The general method for detecting compound X chromosomes is to mate a female, heterozygous for the chromosomes to be attached, to a male carrying some sex-linked dominant marker, as, for instance, *B*. The regular female class will be heterozygous *B*, but there will appear occasional exceptional females, that is, non-*B*. These females carry both X chromosomes from the parental female. Upon testing, some of these will prove to be simple non-disjunctional females, while others may carry the two X chomosomes attached. The type of compound produced will depend, of course, upon the chromosomes carried by

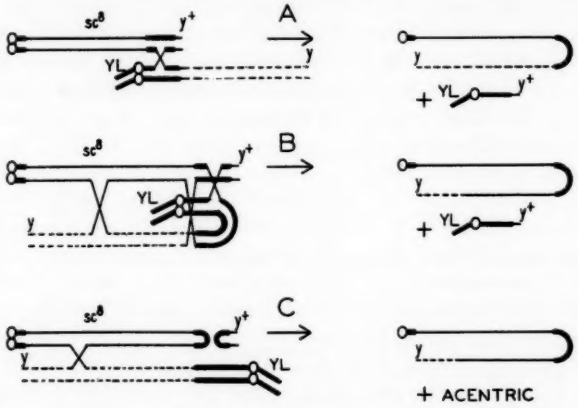

FIGURE 1.—Possible modes of origin of reversed acrocentric compound X chromosomes from a combination of In(1)sc^8 and a chromosome in normal sequence carrying the long arm of the Y chromosome (YL) attached to the centromere. Event C is sister-strand union. It is shown occurring in the distal heterochromatic section of the *sc*^{8} chromosome, but it could presumably occur in the proximal heterochromatic region of the chromosome in normal sequence instead.

the parental female. A method for producing reversed acrocentric compound X chromosomes has been described by NOVITSKI (1954). It consists of attaching In(1)sc^8 (a long inversion of the X chromosome which places a considerable section of the basal heterochromatin including *bb* and Block A distally) and a chromosome in normal sequence carrying appropriate markers. The simplest way in which such an attachment might occur is shown in line A of figure 1. Since the compounds produced can be shown to be hemizygous for the *y* locus, it appears as though the attachment occurs in such a way as to exclude both the centromere region of the chromosome in normal sequence and the distal uninverted section of the *sc*^{8} chromosome from the resulting compound. The results from four experiments designed to produce reversed acrocentrics are given in table 1. The genetic constitution of twelve of the fifteen compounds recovered was determined immediately after the compounds were obtained. The results of these determinations are given in table 2.

TABLE 1

The results from four crosses made for the purpose of detecting spontaneous reversed acrocentric compound X chromosomes. The parental males in every case carried the attached XY (= YSX.YL), yB chromosome. Experiment II, and all subsequent data from lines ND1 and ND9, are taken from Novitski (1954).

Experiment number	Constitution of parental ♀	Number of regular ♀♀	Exceptional females		
			Non-disjunctional	Sterile	Compound
I	$\frac{sc\ cv\ v\ f\ car}{sc^8}$	15,575	4	0	0
II	$\frac{sc\ cv\ v\ f\ (C\text{-}2).YL}{sc^8}$	8,897	0	5	4
III	$\frac{sc\ cv\ v\ f.YL}{sc^8}$	9,972	5	3	5
IV	$\frac{y\ cv\ v\ f\ car.YL}{sc^8}$	35,836	2	3	5*

*** One of the females included in this figure was lost before tests could be made. It is included here as a compound because it was phenotypically *y cv v f car*.**

Although it is true that theoretically other kinds of compounds (e.g., attached-X's) could be produced from these experiments, it is possible, upon testing, to distinguish reversed acrocentrics from these (see below). All of the compounds which were tested have proved to be reversed acrocentrics.

Event giving rise to reversed acrocentrics

Since homozygosis for loci in reversed acrocentrics requires a double exchange or one of higher rank (MULLER 1943), and since, as will be shown below, the frequency of homozygosis in reversed acrocentrics is low in general, it seems most probable that the homozygosis exhibited by the seven of the

TABLE 2

The genetic constitution of females carrying newly formed reversed acrocentric compound X chromosomes. The Roman numerals in parentheses refer to the experiment number as given in table 1.

Line	Parental female heterozygous for		Constitution of compound
ND1	*cv v f*	(II)	*cv/+, v/+, +/+*
ND9	*cv v f*	(II)	*+/+, +/+, +/+*
ND18	*cv v f*	(III)	*cv/+, v/+, f/+*
ND23	*cv v f*	(III)	*cv/+, v/+, f/+*
ND25	*cv v f*	(III)	*cv/+, v/+, f/f*
ND27	*cv v f*	(III)	*cv/+, v/v, f/f*
ND28	*cv v f*	(III)	*cv/+, v/+, f/+*
ND33	*cv v f car*	(IV)	*cv/+, v/+, f/f, car/car*
ND34	*cv v f car*	(IV)	*cv/+, v/+, f/+, car/+*
ND35	*cv v f car*	(IV)	*cv/cv, v/v, f/f, car/car*
ND36	*cv v f car*	(IV)	*cv/+, v/v, f/f, car/car*
ND37	*cv v f car*	(IV)	*cv/+, v/+, f/+, car/+*

twelve tested compounds (see table 2) occurred coincidentally with their formation. If the compounds were formed in the manner indicated by line A of figure 1, it would require at least a double exchange in addition to the heterochromatic exchange giving rise to the compound to account for this homozygosis. The heterochromatic exchange, furthermore, would have had to take place in a region which was paired in an inversion configuration. Such an event is diagrammed in line B of figure 1. In each instance, moreover, were this the case, one of the exchanges responsible for the homozygosis would have had to take place between the marker nearest the interstitial heterochromatin (*f* in experiments II and III; *car* in experiment IV) and the exchange giving rise to the compound. This is so for the reason that when any locus was homozygous, all of the loci between that one and the interstitial heterochromatin were also homozygous (see table 2). That such a high proportion of the compounds would be produced by triple exchanges having a non-random distribution along the length of the chromosome seems extremely unlikely, and it appears more reasonable, therefore, to assume that reversed acrocentrics arise in two distinctly different ways: (1) by a simple exchange in the heterochromatic regions of the chromosomes (as shown in line A of figure 1) giving rise to those compounds heterozygous for all of the markers which were heterozygous in the parental female; and (2) by a more complicated event which may be thought of simply as the union of sister chromatids in the heterochromatin, plus a single exchange in the euchromatin (as diagrammed in line C of figure 1) giving rise to those compounds homozygous for all of the loci between the euchromatic single exchange and the interstitial heterochromatin. This sister-strand union can apparently occur either in the distal heterochromatic section of the sc^8 chromosome or in the proximal heterochromatin of the chromosome in normal sequence, since some of the compounds recovered were homozygous for alleles carried on the sc^8 chromosome and others were homozygous for alleles on the chromosome in normal sequence.

Another argument can be applied in support of the assumption that compounds showing homozygosis at the time of their origin arise from sister-strand union. Since the base of the sc^8 chromosome, which provides the centromere region of the compounds, lacks the *bb* locus and the nucleolus organizing region (*NO*), these two regions must be present in the interstitial heterochromatin of the recovered compounds for the following reason. The parental males carried the attached XY (= YSX.YL) chromosome (LINDSLEY and NOVITSKI 1950), which is a single chromosome containing the essential elements of the X chromosome plus the fertility factors of the Y chromosome. The compound-bearing females recovered, consequently, did not carry a Y chromosome which would cover heterochromatic deficiencies. Therefore, if a compound arises from sister-strand union, the event would have to take place distal to *bb* and *NO* in the sc^8 chromosome or proximal to these regions in the chromosome in normal sequence in order for the compound-bearing females to be viable. Experiment II, table 1, is of special interest in this connection. The chromosome in normal sequence carries the C-2 deficiency (NOVITSKI

1952), which is a deficiency for some of the basal X chromosome heterochromatin including *bb* and presumably *NO*. Because this is the case, sister-strand union in this chromosome could not give rise to viable products. Since, in experiment II, the C-2 chromosome carried the mutant alleles in the parental female, any compounds recovered from this cross which showed homozygosis would have had to be homozygous for wild-type alleles only, if the assumption of sister-strand union is correct. Two of the compounds recovered from this cross were tested. One of these (ND1) was homozygous for the wild-type allele of *f;* another (ND9) was homozygous for the wild-type alleles of *cv, v* and *f;* and there were four others which, although they were not tested sufficiently to determine which markers were heterozygous, nonetheless were not homozygous for mutant alleles.

Still another argument in favor of the view that compounds showing homozygosis at the time of their origin result from sister-strand union is as follows. If the event giving rise to the compounds were an ordinary exchange between the distal heterochromatin of the sc^8 chromosome and the proximal heterochromatin of the chromosome in normal sequence, there would necessarily be produced, as a complementary product, a chromosome fragment carrying the centromere of the chromosome in normal sequence (which has the long arm of the Y chromosome attached to it), plus the uninverted section of the sc^8 chromosome including the normal allele of *y* (see lines A and B of figure 1). Irrespective of whether this event is meiotic or mitotic, the fragment should be recoverable either in the compound-bearing females or in patroclinous males. The latter type would be detectable as non-*y* (and hence fragment-bearing), *B* males; the former type of course would have routinely been tested, since they would be exceptional females. Two such fragments have been reported from experiments similar to those described here (NOVITSKI 1954), but no such fragments have been recovered from experiments III and IV, although from these same experiments a total of ten compound-bearing females were recovered.

This discrepancy between the number of fragments recovered as compared with the number of compounds recovered is consistent with the assumption that sister-strand union is responsible for a large proportion of the compounds, since under this assumption the fragments would be eliminated as acentrics (see line C of figure 1), and is inconsistent with the alternative that simple exchange in the heterochromatic regions of the chromosomes to be combined accounts for most of the compounds.

Further information relative to sister-strand union

In all of the experiments described above, the only place sister-strand union could have occurred and given rise to recoverable products was in the heterochromatic regions such that *bb* and *NO* were included in the resulting compounds. In order to test whether the event is limited to these regions or not, it becomes necessary to be able to recover compounds carrying homozygous deficiencies for regions in and around the interstitial heterochromatin. For this

purpose the following cross was made. Females heterozygous for In(1)sc^8, *cv v f* and a chromosome in normal sequence carrying the markers *y w*, the *C-2* deficiency (for description see above), and the long arm of the Y chromosome attached to the centromere (= *sc^8, cv v f/y w (C-2)*.YL) were mated to males carrying T(1,4)B^8. The right end of the *B^8* translocation is a chromosome fragment consisting of the centromere of the X chromosome, all of the basal heterochromatin, and the euchromatin up to and including the *B* locus. The fragment is marked by the dominant allele *B*. In this cross, compound-bearing females could be recognized since they would be phenotypically *y*. Compounds which were deficient for any of the heterochromatic regions, or euchromatic regions adjacent to the interstitial region, would be recoverable when fertilization was effected by a sperm carrying the *B^8* duplication. Compounds which did not receive the *B^8* duplication would receive a Y chromosome and might therefore still be recoverable as homozygous deficiencies for heterochromatic regions.

TABLE 3

The results of tests of y (and, hence, compound-bearing) female progeny from a mating of yw(C-2).YL/sc^8, cv v f ♀♀ × T(1,4)B^S ♂♂. The total of ten lines includes one cluster of four (ND39) and one cluster of two (ND41).

Line	Phenotype when obtained	Constitution	Viable as XX/Y	Viable as XX/O
ND39a	*y B^S*	Sterile	?	?
ND39b	*y B^S*	*cv/+, v/+, +/+*	+	–
ND39c	*y*	Sterile	+	?
ND39d	*y*	Sterile	+	?
ND40	*y*	*cv/+, +/+, +/+*	+	–
ND41a	*y B^S*	*cv/+, v/+, +/+*	+	–
ND41b	*y*	*cv/+, v/+, +/+*	+	–
ND42	*y f*	*cv/+, v/+, f/f*	+	+
ND43	*y f*	Sterile	+	?
ND44	*y v f*	*cv/+, v/v, f/f*	+	+

The progeny from approximately 450 matings were examined (no counts were made). This represents an experiment approximately twice the size of experiment IV, table 1. Six separate compounds were recovered from this experiment, two of which, however, occurred as clusters of identical individuals. Tests were carried out on all of these compounds. The results of these tests are summarized in table 3. Those compounds which were inviable in the absence of a Y chromosome (as XX/O) were tested for viability with a chromosome fragment equivalent to the long arm of the Y (= Y^{cl}). None of them were viable with this chromosome fragment. These compounds, therefore, must lack some factor necessary for survival which can be supplied by the short arm of the Y chromosome. Since all of the compounds were homozygous for loci adjacent to the interstitial heterochromatin, it appears as though all of these resulted from sister-strand union.

From these data, there are three points of interest. First, since all of the fertile compounds were viable with a Y chromosome and therefore carried no

euchromatic deficiencies, it would seem as though sister-strand union in euchromatic regions is at least as rare as sister-strand union in heterochromatic regions. Secondly, in this cross the C-2 chromosome carried the wild-type alleles of the markers used, and hence, compounds which were homozygous for wild-type alleles would be expected to be lethal without a Y chromosome according to the assumption of sister-strand union since they should lack the *bb* locus and *NO*. Those compounds which were homozygous for mutant alleles (and therefore had interstitial heterochromatin derived from the sc^8 chromosome) might be expected to be viable under the same condition. Table 3 shows that this is, in fact, the case, and hence this experiment provides the most convincing evidence for sister-strand union thus far.

Finally, it should be noted that ND39 and ND41 each occurred as a cluster. Since the frequency of reversed acrocentric formation is very low, the occurrence of a cluster of compound-bearing individuals provides very strong evidence that these compounds arose during the gonial divisions; that is, a single compound was formed in some pre-meiotic mitosis, resulting, as a consequence of subsequent mitotic divisions, in a group of cells each carrying this compound.

Effect of YL on reversed acrocentric formation

Experiment I, table 1, differed from the other experiments designed to produce reversed acrocentric compounds in that the chromosome in normal sequence lacked the long arm of the Y chromosome (YL) attached to the centromere. From this experiment no compounds were recovered although the total number of flies examined was greater than for experiments II and III from which compounds were obtained. These data suggest the possibility that a Y chromosome arm at the base of one of the chromosomes to be attached is necessary for the production of reversed acrocentrics. In order to test this point, five different chromosome combinations which might be expected to yield reversed acrocentric compounds, but in which a Y chromosome arm was not involved, were tested in much the same manner as were the sc^8/normal combinations described above. No compounds were recovered from a total of 43,379 female progeny examined. It appears most likely, therefore, that Y chromosome heterochromatin at the base of one of the chromosomes to be attached is necessary for the production of reversed acrocentric compounds. It should be noted that there is no simple scheme whereby YL could be directly involved in the exchange giving rise to the compound, as such an exchange would ordinarily lead to the production of a dicentric.

MEIOTIC BEHAVIOR OF REVERSED ACROCENTRICS

Meiotic loss of unpaired reversed acrocentrics

Since it has been shown previously (SANDLER and BRAVER 1954) that chromosomes in Drosophila which fail to pair with a homolog may be lost during the meiotic divisions, it becomes of interest (both in its own right and for purposes of the analysis to follow) to determine whether or not reversed

TABLE 4

The results from a series of parallel matings of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of y and heterozygous for In(1)AB and v, both with and without a pairing partner (=FR2), to YSX.YL, y B males both with and without FR2. The compound-bearing females are symbolized as y; the YSX.YL, y B males as X-Y.

Cross	Regular		Non-disjunctional		(y or y^+)v ♀♀
	♀♀	♂♂	♀♀	♂♂	
y × X-Y	1,944	1,851	....	....	13
y/FR2 × X-Y	3,644	3,057	6	47	8
y × X-Y/FR2	1,824	2,175	0	1	5
y/FR2 × X-Y/FR2	3,790	3,593	....	97	17

acrocentrics undergo meiotic loss. For this purpose, a series of crosses was made using females carrying reversed acrocentric compounds hemizygous for the mutant allele of *y*, heterozygous for In(1)AB (a small inversion extending from near *v* to between *g* and *sd*) and *v*, both with and without FR2 (NOVITSKI 1952), by *y B* males carrying the YSX.YL chromosome both with and without FR2. FR2 is a heterochromatic fragment consisting of the long arm of the Y chromosome, and is marked by the normal allele of *y* from In(1)sc^8. The results from this series of experiments are given in table 4. Since In(1)AB is inseparable from the normal allele of *v*, females which lose the inversion by a double crossover are recognizable as homozygotes for the mutant allele of *v*. Females of this type were collected and used in a series of experiments identical with that described above. The results from this series of experiments are given in table 5. The relevance of these two series, in connection with the calculation of exchange values, is shown below. From the frequency of the exceptional classes it can be seen that FR2 provides a homolog for both the reversed acrocentric compound and the YSX.YL chromosome. As a consequence, the disjunctional behavior of both of these chromosomes can be studied with and without a pairing partner.

There are, in each set of experiments, two comparisons that can be made to obtain the frequency of meiotic loss for the compound chromosome, and two comparisons for the loss of the YSX.YL chromosome. For loss of reversed

TABLE 5

The results from a series of parallel matings of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of y and homozygous for v, both with and without a pairing partner (=FR2) to YSX.YL, y B males both with and without FR2. The compound-bearing females are symbolized as yv; the YSX.YL, y B males as X-Y.

Cross	Regular		Non-disjunctional	
	♀♀	♂♂	♀♀	♂♂
yv × X-Y	945	1,352	....	....
yv/FR2 × X-Y	2,441	2,806	16	51
yv × X-Y/FR2	513	945	0	0
yv/FR2 × X-Y/FR2	2,001	3,161	....	81

acrocentrics the first two lines in tables 4 and 5 give a comparison in which the male is a constant, while the compound in one case has a homolog (FR2) and in the other case has not, and a similar comparison is afforded by the last two lines in each table. For loss of the YSX.YL chromosome, the first and third lines of each table represent cases in which the female is a constant, while the YSX.YL chromosome in one case has a homolog and in the other case has not, and the other comparison of this type is between the second and fourth lines in each table. Loss is calculated as the percentage discrepancy between the two experiments compared; that is, 100% – (observed ratio without homolog/observed ratio with homolog).

For the experiments in which the reversed acrocentrics were heterozygous for In(1)AB, loss of the compound chromosome was 12 percent in one case, 100% – (1,944/1,851)/(3,644/3,057), and 20 percent in the other case, 100% – (1,824/2,175)/(3,790/3,593). Loss of the YSX.YL chromosome was 20 percent in the first comparison, 100% – (1,851/1,944)/(2,175/1,824), and 12 percent in the other, 100% – (3,057/3,644)/(3,593/3,790). Similar calculations for the set in which the compounds were not heterozygous for an inversion gives the following results. Loss of the compound chromosome was 20 percent in the first case, 100% – (945/1,352)/(2,441/2,806), and 14 percent in the other case, 100% – (513/945)/(2,001/3,161). Loss of the YSX.YL chromosome was 22 percent in the first comparison, 100% – (1,352/945)/(945/513), and 27 percent in the other, 100% – (2,806/2,441)/(3,161/2,001).

Although the magnitude of the discrepancy varies somewhat from experiment to experiment, the data indicate that the reversed acrocentric is lost in 12 to 20 percent of all cases, when it has no homolog.

Exchange in reversed acrocentrics

The genetically distinct types of exchanges in the reversed acrocentric compound X chromosome are shown in figure 2. The genetic consequences of all combinations of these exchanges are given in table 6. Extensive tests were

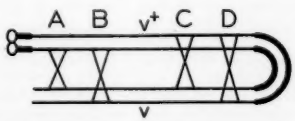

FIGURE 2.—The genetically distinct types of exchanges in the reversed acrocentric compound X chromosome. The consequences of all possible combinations of these exchanges are given in table 6.

made of lines ND1, ND18, ND23, ND33, ND34 and ND37. In certain of these tests the females carried FR2, while in others the females did not. The males in every case carried the YSX.YL, *y B* chromosome. The results from these crosses are given in tables, 7, 8, 9 and 10. Table 11 gives a summary of the frequencies of homozygosis for all of the tests made.

From the data presented, a number of points of interest arise. First, there

TABLE 6

The consequences of exchange of ranks 0, 1, and 2 in the reversed acrocentric compound X chromosome. Those exchanges which produce products not different from the ones given in the table have been omitted when the omission does not alter the relative frequencies of products from the various types of exchanges. E_0 = no-exchange; E_1 = single exchange; and E_2 = double exchange. The "crossovers involved" are shown in figure 2.

Rank and frequency	Crossovers involved	Products after anaphase II separation	
E_0	None	Reversed acrocentric + reversed acrocentric	
		(*v*/+)	(*v*/+)
$1/2E_1$	A or D	Reversed acrocentric + reversed acrocentric	
		(*v*/+)	(*v*/+)
$1/2E_1$	B or C	Bridge	
$1/4E_2$	A and C	Reversed acrocentric + reversed acrocentric	
		(*v*/*v*)	(+/+)
$1/4E_2$	A and D	Reversed acrocentric + reversed acrocentric	
		(*v*/+)	(*v*/+)
$1/4E_2$	B and C	Reversed acrocentric + reversed acrocentric	
		(*v*/*v*)	(+/+)
$1/4E_2$	B and D	Bridge	

is an unexpectedly high frequency of homozygosis for loci adjacent to the interstitial heterochromatin (*f* and *car*) as compared with the frequencies obtained from simple attached-X experiments. Secondly, in the presence of FR2 the frequency of homozygosis in the compound chromosome is markedly increased. Finally, in the case of ND33, ND34 and ND37 there appears to be no appreciable reduction in the number of females, although from table 6 it

TABLE 7

The results from crosses of females, each of which carries a reversed acrocentric compound X chromosome, to yB males carrying the YSX.YL chromosome. ND18 and ND23 are heterozygous for cv, v, and f; the parental females carry a heterochromatic fragment marked by the normal allele of y (=FR2). ND1 is heterozygous for cv and v only, and the parental females did not carry FR2.

Phenotype of progeny	ND1	ND18	ND23
+ ♀♀	1,479	2,320	2,327
regular ♂♂	1,974	3,412	3,568
cv ♀♀	4	6	52
v ♀♀	27	77	106
f ♀♀	....	65	84
cvv ♀♀	5	23	0
vf ♀♀	...	67	73
cvvf ♀♀	...	15	0
exceptional ♂♂	....	55	101

TABLE 8

The results from a mating of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of y, homozygous for the mutant alleles of f and car, and heterozygous for cv and v, to YSX.YL, y B males. The results given in the second column are from females which carried a heterochromatic fragment marked by the normal allele of y (=FR2) in addition to the compound.

Phenotype of progeny	ND33	ND33/FR2
y f car ♀♀	959	2,235
Regular ♂♂	1,105	2,281
y cv f car ♀♀	11	64
y v f car ♀♀	31	194
y cv v f car ♀♀	1	4
Exceptional ♀♀	....	11
Exceptional ♂♂	....	52

can be seen that one half of the single exchanges plus one quarter of the double exchanges should produce anaphase II bridges which would cause a reduction in the female class (STURTEVANT and BEADLE 1936). Each of these points will be considered separately.

Crossing over in the interstitial region

Reversed acrocentrics differ structurally from simple attached-X chromosomes primarily with respect to the position of the centromere; it being subterminal in the case of reversed acrocentrics and median in the case of the attached-X. The basal heterochromatin of attached-X chromosomes, therefore, is analogous to the interstitial heterochromatin of reversed acrocentrics. With respect to this comparison, it seems most likely that attached-X chromosomes must have, at the very least, as much or more basal heterochromatin than reversed acrocentrics have interstitial heterochromatin, for the reason that an attached-X presumably possesses two complete basal regions, while the inter-

TABLE 9

The results from a mating of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of y and heterozygous for cv, v, f, and car, to YSX.YL, y B males.

Phenotype of progeny	ND34	ND37
y ♀♀	863	694
y B ♂♂	966	849
y cv ♀♀	3	3
y v ♀♀	8	8
y f ♀♀	3	3
y car ♀♀	1	0
y cv v ♀♀	1	8
y v f ♀♀	3	2
y f car ♀♀	16	12
y cv v f ♀♀	1	0
y v f car ♀♀	1	3
y cv v f car ♀♀	0	0

TABLE 10

The results from a mating of females, each of which carries a reversed acrocentric compound X chromosome hemizygous for the mutant allele of y and heterozygous for cv, v, f, and car, plus a heterochromatic fragment marked by the normal allele of y (=FR2), to YSX.YL, yB males.

Phenotype of progeny	ND34	ND37
y ♀♀	2,162	1,886
B ♂♂	2,457	2,279
ycv ♀♀	7	15
yv ♀♀	92	76
yf ♀♀	31	28
ycar ♀♀	2	17
ycvv ♀♀	48	36
yvf ♀♀	32	38
yfcar ♀♀	71	42
ycvvf ♀♀	6	8
yvfcar ♀♀	28	27
ycvvfcar ♀♀	9	13
yB ♂♂	43	40
Exceptional ♀♀	3	14

stitial heterochromatin of reversed acrocentrics is composed of some fraction of two complete basal regions (see fig. 1). Homozygosis for loci in both of these compounds requires a crossover between the locus and the heterochromatic region, although an additional exchange to the other side of the locus is necessary in the case of reversed acrocentrics. Homozygosis for *car* in attached-X experiments normally runs at less than one percent, whereas in the case of reversed acrocentrics, as can be seen from table 11, it ranges from 4.26 percent to 4.53 percent when the parental females carried FR2, and 2.00 percent to 2.05 percent when the parental females did not carry FR2. Since this difference apparently cannot be a function of the length of the heterochromatic regions involved, it would appear to be some function of the position of the locus with respect to the position of the centromere. Suppression of crossing over as a consequence of the proximity of the centromere has been reported

TABLE 11

A summary of the frequencies of homozygosis for mutants in reversed acrocentric compound X chromosomes. The experiment number refers to table 1. The figures presented are calculated from the data given in tables 7, 8, 9, and 10.

Line	Experiment number	% homozygosis			
		cv	*v*	*f*	*car*
ND1	II	0.59	2.11	....	....
ND18/FR2	III	1.71	7.07	5.71	....
ND23/FR2	III	1.97	6.78	5.94	....
ND33	IV	1.20	3.19	....	....
ND33/FR2	IV	2.72	7.93	....	....
ND34	IV	0.56	1.56	2.56	2.00
ND34/FR2	IV	2.81	8.64	7.11	4.26
ND37	IV	1.50	2.86	2.73	2.05
ND37/FR2	IV	3.29	9.06	7.14	4.53

previously for other situations in Drosophila (BEADLE 1933), and is known generally as the centromere effect.

Effect of FR2 on crossing over

From the crossover data presented it can be seen that the experiments fall into two distinct classes; those in which the females carried FR2, giving crossover values ranging from 13.7 percent (ND37/FR2) to 9.8 percent (ND18/FR2), and those in which the females carried no pairing partner for the reversed acrocentric, giving crossover values ranging from 5.3 percent (ND37) to 2.4 percent (ND1). The best comparisons come, of course, from lines ND33, ND34 and ND37 for which there are parallel experiments both with and without FR2. It is clear that the fragment has the effect of approximately doubling the rate of homozygosis. It should be noted, however, that the relative proportions of the F_1 classes showing homozygosis for markers near the interstitial region appears to be higher in those cases in which the parental females did not carry FR2.

Certain other information relevant to this effect has been collected. FR2 consists mainly of the heterochromatin from the long arm of the Y chromosome, and it might be wondered, therefore, whether the increase in homozygosis is simply a consequence of the presence of a heterochromatic fragment, or whether the effect is due particularly to Y chromosome heterochromatin. In order to test this point, females carrying reversed acrocentrics hemizygous for the mutant allele of *y*, heterozygous for *v*, and carrying a free fragment consisting of X chromosome heterochromatin (the right end of $T(1,4)B^8$; for description see above) were crossed to YSX.YL, *y B* males. The progeny included 1,325 *y* ♀♀; 455 *y B*/B^8 (hyperploid) ♂♂; 30 *y v* ♀♀; 58 exceptional females (one of which was *v*); and 130 exceptional males. This represents a frequency of homozygosis for *v* of 2.21 percent (30/1,355). From these data it appears, then, that the effect of increasing crossing over does not extend to X chromosome heterochromatin.

It becomes of interest, consequently, to determine whether this effect is confined to the compound X chromosome, or whether it is a general effect extending to all of the chromosomes in the complement. To test this, females carrying reversed acrocentrics hemizygous for the mutant allele of *y*, heterozygous for *v* and the third chromosome dominants D^3 and *H*, and carrying the sc^8.Y chromosome (MULLER 1948) were crossed to YSX.YL, *y B* males. The sc^8.Y is a full Y chromosome marked by the normal allele of *y*. The frequency of homozygosis for *v* in this experiment was 9.60 percent (117/1,218), and there was 28 percent crossing over between D^3 and *H*. From among the progeny of this cross *y*, non-*v*, D^3 *H* females were collected and crossed to YSX.YL, *y B* males. These females are identical with the ones used in the experiment described above except that they do not carry a Y chromosome. Homozygosis for *v* in this case was 4.48 percent (16/357), and there was 29 percent crossing over between D^3 and *H*.

From these data it would appear that the effect of a Y chromosome on

crossing over is confined to the compound X chromosome. It might in addition be noted that these results also show that the effect is produced by a full Y chromosome as well as by FR2.

Analysis of exchange with respect to rank

The frequencies of no, single and double exchanges for any test can be arrived at from the frequency of homozygosis and the discrepancy between males and females. For example, in the case of ND34 carrying FR2 (table 10) there were a total of 2,488 regular females and 2,457 regular males. Since meiotic loss of the YSX.YL chromosome results in a 16 percent deficiency (the average loss of this chromosome as measured in a number of different experiments) of the male class, the males must be increased by that amount. This means that there should actually have been 2,925 (2,457/0.84) males. The observed frequency of homozygosis equals 13.1 percent (326/2,488). This represents a total frequency of homozygosis of 26.2 percent since only one half of the homozygotes are homozygous for mutant alleles. The total frequency of double exchange is equal to twice the frequency of homozygosis (see table 6) or 52.4 percent. One quarter of these doubles produce anaphase II bridges which are lethal and hence cause a corresponding depression in the female class. This amounts to a 13.1 percent depression of females attributable to lethal bridges coming from double exchanges. The total observed depression in the female class amounts to 14.9 percent (2,925 – 2,488/2,925), and hence the anaphase II bridges that come from single exchanges equals 1.8 percent (14.9% – 13.1%). One half of all singles should produce lethal bridges, and therefore the total frequency of singles becomes 3.6 percent. Since doubles equal 52.4 percent, and singles equal 3.6 percent, no-exchange tetrads must have a frequency of 44.0 percent.

A similar analysis for ND37/FR2 (table 10) gives the following results: no-exchange = 33.8%; singles = 11.4%; and doubles = 54.8%. For ND34 not carrying FR2 (table 9), if it is assumed that meiotic loss is the same for the compound X chromosome as for the YSX.YL chromosome (and may therefore be ignored), no-exchange = 78.2%; singles = 5.4%; and doubles = 16.4%. For ND37 not carrying FR2 (making the same assumption about meiotic loss) no-exchange = 62.2%; singles = 16.6%; and doubles = 21.2%.

There are two points which should be noted with respect to this analysis. First, it is assumed that the anaphase II bridges produced by crossing over are lethal. Evidence for this is found in tables 4 and 5, from which it can be seen that the frequency of the exceptional (that is, *y B*) male class is approximately the same, irrespective of whether the compound was heterozygous for an inversion or not. The comparisons come from those cases in which the compound-bearing females carried FR2. The frequency of exceptional males from females heterozygous for the inversion is 2.1 percent (144/6,894), and the frequency from females not heterozygous for the inversion is 2.2 percent (132/6,099). The reduced frequency of homozygosis for *v* in those compounds heterozygous for the inversion indicates that the rate of crossing over is very much reduced.

If the anaphase II bridges were not lethal, but instead were lost to the daughter nuclei (as are anaphase I bridges), then there would be produced nullo-X eggs which, after fertilization by YSX.YL, *y B* sperm, would produce exceptional males. Since the frequency of these males does not vary with crossing over, it seems fairly certain that the anaphase II bridges, when produced, are lethal. The second point to note is that any reasonable assumption about meiotic loss cannot account for the reduced frequency of single exchanges, since not assuming meiotic loss would result in a lower calculated frequency of singles, and the frequency of loss of the YSX.YL chromosome necessary to bring the singles up to where they would compare reasonably with the doubles (the calculated frequency of which is independent of either anaphase II bridges or meiotic loss) is much higher than anything ever observed for this chromosome.

From this analysis it appears as though single exchanges are very infrequent as compared with double exchanges. This estimate, however, comes from that half of the singles which produce lethal anaphase II bridges. The other half of the singles, which correspond to exchange A or D in figure 2, have the effect only of changing the position of the mutants with respect to the strand they are on. For example, a compound symbolized *cv v f car*/++++, with a single exchange of the type not producing an anaphase II bridge between *v* and *f*, would produce, as one of the products, a compound which could be symbolized *cv v* ++/++ *f car*. This is not an immediately detectable product, but an estimate of the frequency of this can be arrived at in the following manner. If two mutants are separated by a single exchange and displaced to different strands with respect to each other (as indicated in the above scheme), then it requires at least a quadruple exchange to produce a single individual which is homozygous for both of these mutants. It is therefore possible to arrive at a maximum estimate for the frequency with which such an exchange has occurred simply by examining the progeny of single females and checking for those cases in which adjacent mutants have not appeared simultaneously. This would be a maximum estimate for the reasons that (a) two-strand doubles with one exchange between *car* and the interstitial region, and one half of all four-strand doubles would produce products identical with those produced by this type of single, and (b) the overall frequency of homozygosis being low, the critical types might not appear among the progeny simply by chance. It should be noted, moreover, that there is no selection against compounds resulting from a single exchange of this type, and, as a consequence, they should accumulate in stock. The tests recorded in table 10 were made three generations after the compounds were obtained, and single females were used as parents. The progeny of each individual female was examined, and those cases selected in which all of the mutants appeared at least once. Of the 40 females of this type whose progeny was examined, 35 must have been *cv v f car*/++++, while five could have had mutants displaced with respect to strand. This number must be doubled ,since only one of the products from a tetrad with the type of single not producing an anaphase bridge will have mutants displaced. This represents a maximum frequency of this event of 25 percent ($2 \times 5/40$) over three genera-

tions. The overall frequency per generation, then, is 8.33 percent (0.25/3); a value which is in agreement with that found in the analysis given above in that they both show surprisingly low values for single exchanges.

The expectations from exchanges of rank 3 are: compounds not showing homozygosis = 1/8; compounds showing homozygosis (total) = 4/8; anaphase II bridges = 3/8. Since the frequency of homozygosis remains the same as for double exchanges, and the frequency of bridges goes up, the analysis given above would admit of some frequency of triple exchanges, but since the frequency of singles is taken from the lethality of anaphase bridges, any frequency of triples would detract from the calculated number of singles.

The analysis thus far has been confined to lines ND33, ND34, and ND37 for the reasons that (a) in these lines there were parallel sets both with and without FR2; (b) these lines were marked by the mutant allele of *y*, and, in the case of ND34 and ND37, were heterozygous for *car;* and finally (c) the other lines, that is ND1, ND18, and ND23, were tested before it was known that the YSX.YL chromosome would be lost in the absence of a homolog, and therefore no special effort had been made to insure the absence of a free Y chromosome in the male stocks. However, evidence that all the lines behave in the same way comes from table 11, from which it can be seen that the frequencies of homozygosis are roughly the same for all of the comparable tests. In addition, ND23 is of interest because it can be seen (from table 7) that all of the females tested (there were 40) must have been of the constitution *cv v* +/+ + *f*. This test was made three generations after the original compound-bearing female was obtained, and hence it appears that a single exchange (or two-strand or four-strand double) between *v* and *f* occurred once, some time previous to the test, and that all compounds tested subsequently from this line were of this constitution.

It appears from the data then, that single exchanges in reversed acrocentric compounds are extremely rare with respect to doubles; that is, most tetrads have either no exchange or a multiple exchange.

DISCUSSION

The information that a Y chromosome or a Y chromosome fragment can approximately double the rate of homozygosis in reversed acrocentrics is of some interest with respect to the pairing properties of this particular type of compound. First, since this effect is not a general one for the entire chromosome complement but is confined to the compound chromosome, and, since the homology between the compound chromosome and the Y chromosome is indicated by the regular disjunction of each from the other, it seems likely that the effect of the Y chromosome on the rate of homozygosis is related to the pairing of these two chromosomes. Moreover, since there is no such effect evident from attached-X studies, it seems reasonable to suppose that the effect is in some way related to the position of the centromere in the reversed acrocentric compound. The effect might come about in the following manner. If the spatial relationships between the chromosomes are maintained, to some extent, from

any telophase to the following prophase, then at the first meiotic prophase the reversed acrocentric compound would lie straightened out as a consequence of the anaphase movement of the preceding mitosis. If such were the case, then, in order for the components of the compound chromosome to synapse with each other, the compound would have to fold back on itself; a movement which would not be required in the case of the attached-X. It is conceivable that such folding back would be facilitated by a heterochromatic fragment, perhaps by pairing with the interstitial region and thereby mechanically causing the folding back. The fact that homozygosis for markers near the interstitial region was relatively higher when the parental females did not carry FR2 may well be evidence in support of the view that the fragment does pair interstitially with an appreciable frequency. This sort of notion requires, however, that Y chromosome heterochromatin have a different homology with X chromosome heterochromatin than X chromosome heterochromatin has with itself, since the effect is caused specifically by Y chromosome heterochromatin.

It has been shown that a simple analysis of crossover data from reversed acrocentrics indicates that there is a decided deficiency of single, as compared with double, exchanges. The example worked out in text gives the following values for the frequencies of tetrads of different ranks: no-exchange = 44.0%; single exchange = 3.6%; and double exchange = 52.4%. Data indicating that such a distribution of exchanges is atypical for X chromosomes in Drosophila is available from other sources. For instance, exchange values for unattached X chromosomes heterozygous for *sc ec cv ct v s f car bb* have been calculated by WEINSTEIN (1936). The frequencies of tetrads of the different ranks are given as: no-exchange = 5.6%; single exchange = 48.5%; double exchange = 42.9%; and triple exchange = 3.0%. It should be noted that the value given above for single exchanges in reversed acrocentrics is a maximum estimate, and it may very well be that the true value for single exchanges is zero. This is so for the following reasons. The frequency of single exchanges is calculated from the discrepancy between the recovery of males as compared with females, and also (independently) from the cases in which adjacent mutants have been displaced to different strands. It can be seen from the treatment given in text that the first method depends to a large extent upon the estimate of the amount of meiotic loss of the YSX.YL chromosome in the parental male, which, if it were overestimated by just a few percent, would erroneously give the appearance of some frequency of single exchanges. The other method depends upon the identification of chromosomes which are produced by single exchanges, but which are also produced by certain of the two-strand doubles and one half of all four-strand doubles.

Although it is not possible to state categorically why the reversed acrocentric compound should give a value for single exchanges of close to zero, certain possibilities may be eliminated from the data now available. The most obvious of these possibilities is that the participation of the strands in exchange is not at random. This would be chromatid interference in the case of double or higher rank exchanges and "strand preference" in the case of single ex-

changes. Both of these possibilities may be eliminated as being responsible for the observed lack of single exchanges. Since that half of the single exchanges which does not produce anaphase bridges are found to occur with a very low frequency, the only way strand preference could operate would be to give an excess of the other type of single. This type of single produces an anaphase bridge which causes a discrepancy between the recovery of the two sexes, and this discrepancy has not been observed. Chromatid interference, on the other hand, only affects the distribution of multiple exchanges, and could, therefore, only affect the calculated recovery of singles if it reduced the contribution of double exchanges to the anaphase bridge class. Since the total number of anaphase bridges is so small, this could not increase the number of singles to any appreciable extent. Moreover, if this were the case, it would mean invoking not only chromatid interference, but also strand preference, since it would mean that more singles of the type which give anaphase bridges were being produced than the type which do not.

Another possibility is that single exchanges occur with the expected frequency, but for some reason are not recovered. This might happen, for instance, if the single exchanges occurred predominately in the gonial divisions just preceding meiosis. This would result in one of two possibilities: (a) the cells containing the bridges would be eliminated before meiosis; or (b) the bridges would be eliminated, but the cells would go through meiosis to produce nullo-X eggs. The latter case would result in a depression of the female class, and this has not been observed. The former possibility, would, it is true, result in the elimination of the bridges without causing a discrepancy in the sex ratio, but it would not explain the lack of that type of single exchange which does not produce an anaphase bridge, since this type of single would not be selected against.

Single exchange chromatids might also not be recovered if there were a preferential recovery of strands not involved in exchange. This should not, however, affect the detection of anaphase bridges, and may, on these grounds, be eliminated as a possible cause for the lack of detected single exchanges.

In this general connection one final point may profitably be made. The reversed acrocentric compound bears a striking resemblance to a heterozygous long inversion in that the pairing properties of both are of such a nature as to give recognizable crossover products only following a double or higher rank exchange. Although it has been generally assumed that single exchanges occur freely within long inversions, the data from reversed acrocentrics might make one wonder whether this is, in fact, the case. To determine this, using unattached X chromosomes, would require some special genetic test.

SUMMARY

Reversed acrocentric compound X chromosomes are essentially like simple attached-X chromosomes except that the centromere is sub-terminal instead of median. They are produced by attaching In(1)sc^{8} and a chromosome in normal sequence carrying appropriate markers. The simplest way in which such

an attachment might occur is by an exchange between the distal heterochromatin of the sc^8 chromosome and the proximal heterochromatin of the chromosome in normal sequence. However, a large proportion (13 out of 18) of the tested compounds were recovered homozygous for loci adjacent to the point of attachment. Since this appears to negate the above assumption, it is suggested that those compounds which are recovered with homozygous markers arise as the result of a more complicated event which may be thought of simply as the union of sister chromatids in the heterochromatin (sister-strand union) plus a single exchange in the euchromatin. Four lines of evidence in support of this view are presented.

An analysis of exchange in reversed acrocentrics shows that: (1) crossing over in the interstitial region is much higher than that which would be expected on the basis of attached-X studies. This appears to be what is generally termed a centromere effect, and has been reported for other situations in Drosophila. (2) The rate of homozygosis is approximately doubled by the presence of a Y chromosome or a Y chromosome fragment. There appears to be no such effect from a fragment containing X chromosome heterochromatin only, and the effect does not extend to the other chromosomes of the complement, but is confined to the compound X chromosome. A possible significance of this effect with respect to the structure of the reversed acrocentric compound is considered. (3) An analysis of exchanges with respect to rank indicates that most tetrads contain either no exchange or a multiple exchange, with single exchanges being very rare or absent. Various explanations for this are considered.

The results from the reversed acrocentric compound may be considered of particular interest in view of the fact that its synaptic configuration is probably very much like that of a heterozygous long inversion.

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LITERATURE CITED

BEADLE, G. W., 1932 A possible influence of the spindle fibre on crossing over in Drosophila. Proc. Nat. Acad. Sci. **18**: 160–165.

LINDSLEY, D. L., and E. NOVITSKI, 1950 The synthesis of an attached X Y chromosome. Drosophila Inf. Ser. **24**: 84–85.

MULLER, H. J., 1943 A stable double X chromosome. Drosophila Inf. Ser. **17**: 61–62.

1944 High primary non-disjunction of the insertional double-X. Drosophila Inf. Ser. **18**: 57.

1948 The construction of several new types of Y chromosomes. Drosophila Inf. Ser. **22**: 73–74.

NOVITSKI, E., 1952 The genetic consequences of anaphase bridge formation in Drosophila. Genetics **37**: 270–287.

1954 The compound X chromosomes in Drosophila. Genetics **39**: 127–140.

SANDLER, L., and G. BRAVER, 1954 The meiotic loss of unpaired chromosomes in *Drosophila melanogaster*. Genetics **39**: 365–377.

STURTEVANT, A. H., and G. W. BEADLE, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21**: 554–604.

VALENCIA, R. M., H. J. MULLER and J. I. VALENCIA, 1949 Formation of attached X's by reverse crossing over in the heterochromatic regions. Drosophila Inf. Ser. **23**: 99–102.

WEINSTEIN, ALEXANDER, 1936 The theory of multiple-strand crossing over. Genetics **21**: 155–199.

RELATION OF CARBON DIOXIDE, OXYGEN AND LOW TEMPERATURE TO THE INJURY AND CYTOGENETIC EFFECTS OF X-RAYS IN BARLEY[1]

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THE biological effects of X-rays can be profoundly modified by atmosphere and temperature (see review by PATT 1953). The present study is a re-examination of the findings of TASCHER (1929) and STADLER (1931) in which barley seeds irradiated on solid carbon dioxide ("dry ice"—temperature of sublimation – 80°C) tolerated a dose of X-rays roughly two-thirds higher than similar seeds would withstand at room temperature. These workers attributed the greater tolerance to the low temperature.

Oxygen has a distinct effect on the frequencies of genetic changes resulting from ionizing irradiations (see reviews by GILES 1950; PATT 1953). It appeared logical, therefore, to re-examine the above findings to determine if the difference in the tolerance of the seeds was properly attributed to the temperature or whether the atmosphere, either presence of carbon dioxide or absence of oxygen, was a factor in the results as well. Experiments attempting to distinguish between these alternatives are the subject of the present study.

While this study was in progress, two reports (KING and SCHNEIDERMAN 1952; SCHNEIDERMAN and KING 1953) appeared which showed that an increase of carbon-dioxide gas in an otherwise normal atmosphere increased the frequency of irradiation-induced chromosomal aberrations in Tradescantia.

In the present study, irradiation-induced injury, chromosomal-aberration frequency, and mutation frequency were recorded following each treatment. Evidence will be presented to demonstrate that these effects are not correlated under the conditions of the experiment. Of particular significance is the finding that radiation-induced genetic mutation frequencies and chromosomal-aberration frequencies are not correlated. Such evidence appears to support the chromosome reunion hypothesis (SCHWARTZ 1952; BAKER and EDINGTON 1952), i.e., differences in atmosphere and temperature during irradiation appear to affect the amount of restitution of broken chromosomes.

MATERIALS AND METHODS

The experiments were conducted with dormant seeds of a diploid barley, variety Himalaya (C.I. 620). The seeds were as homogeneous as possible

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since they were descended from a single plant. To ensure equal moisture conditions from sample to sample, the seeds were stored in a humidity-controlled desiccator for at least six weeks prior to the treatments.

The seeds were subjected to 15,000 r units of X-ray as they lay at random in a single layer in sealed lucite chambers. The tops of the chambers through which the irradiation passed were ⅛ of an inch in thickness. The X-radiation was applied at 500 r per minute at the surface of the seeds for 30 minutes. The dose was measured by a Victoreen Condenser r-Meter. The radiation was obtained from a beryllium window A.E.G. 50 Machlett X-ray tube operated at 34 KVP and 26 ma. The gases used in the experiments were obtained from commercial cylinders and assayed as follows: carbon dioxide (Walter Kidde Co.), 99.6% CO_2, .3% N_2, .1% O_2; oxygen (Industrial Air Products), 99.6% O_2, .4% N_2.

The gas and low-temperature treatments were applied to the seeds in the lucite chambers. Carbon dioxide and oxygen were pumped through the chambers 30 minutes before X-radiation. Then the gases were sealed inside the chambers during the 30 minutes of irradiation. For the low-temperature treatments, the chambers were on dry ice (ca. $-80°C$) for 30 minutes before and during the X-radiation. For the combined gas and low-temperature treatments, the gases were pumped through the chambers for 30 minutes. The chambers were then sealed off and kept on dry ice for 30 minutes before and during the X-radiation treatment. To determine if the gas or low-temperature treatments had any effect on unirradiated seeds, the seeds were treated with the gases in the chambers for one hour or placed on dry ice for one hour.

The effects recorded in this study included (1) injury, as measured by seedling height (at 12 days) and survival to maturity of X_1 plants, (2) frequencies of chromatinic bridges in the first anaphase of root-tip cells of X_1 seeds, (3) frequencies of heterozygous chromosomal interchanges in pollen-mother cells of X_1 plants, and (4) frequencies of seedling mutations among X_2 populations. Details as to the methods of culturing the plants and obtaining data on the various effects have been reported earlier (HAYDEN and SMITH 1949; CALDECOTT and SMITH 1952a). Data on all effects were gathered from the same sample of seed in at least four tests of each treatment.

The influences of carbon dioxide, oxygen, and low temperature on each radiation effect was compared with the results of X-irradiation in air at room temperature.

The differences between means were tested for significance using the method and tables of DAVIES (1950) designed for percentage data. The differences between any two treatments are indicated as being significant at the 1% or 5% level, where such differences exist.

EXPERIMENTAL RESULTS

The discussion of effects will be limited to those treatments involving X-rays, since there is little evidence of any effects of gas or temperature without the superimposition of radiation (tables 1, 2, 3).

Injury

Data on the influence of carbon dioxide and oxygen applied to dormant barley seeds at room and low temperature (ca. −80°C) on the irradiation-induced injury are presented in table 1. They represent summaries of five experiments conducted in 1951 and 1952. It is apparent that low temperature alone reduced injury as measured by seedling height and survival to maturity. This reduction is significant at the 1% level (table 4). Neither of the gases applied at room temperature or low temperature had any influence on these effects.

Cytogenetic effects

Frequencies of chromatinic bridges in root tip cells of X_1 seeds. The induced chromatinic bridge data presented in table 2 are summaries of at least

TABLE 1

A comparison of the effects of X-raying (15,000 r) dormant seeds of barley in air, CO_2, and O_2 at room temperature (ca. 20°C) and at dry ice temperature (ca. −80°C), as measured by survival to maturity and rate of growth (summary of five tests).

Treatment	Seedling height		Survival to maturity		
	Seeds treated	Height (12 days) cm	Seeds treated	Surviving plants	
	No.	*Av.*	*No.*	*No.*	*%*
X-rayed in air at room temp.	410	6.8	840	516	61
X-rayed in air at dry ice temp.	410	7.8	840	642	76
X-rayed in CO_2 at room temp.	410	6.8	840	538	64
X-rayed in CO_2 at dry ice temp.	410	7.7	840	631	75
X-rayed in O_2 at room temp.	410	6.6	700	462	66
X-rayed in O_2 at dry ice temp.	410	7.5	700	509	73
Not X-rayed, dry ice temp.	410	10.0	840	805	96
Not X-rayed, CO_2 at room temp.	210	10.4	840	801	95
Not X-rayed, O_2 at room temp.	410	10.5	700	675	96
Control	410	10.2	840	807	97

four experiments for each treatment. Photomicrographs of bridges similar to those recorded in this table have been published by CALDECOTT and SMITH (1952b).

Each of the gases applied at room temperature increased the induced bridge frequency by about 30% over that produced by X-radiation in air at room temperature. The differences are significant at the 1% level (table 4).

Low temperature reduced the induced chromatinic bridge frequency about 20% below that for X-radiation in air at room temperature. This difference is significant at the 5% level (table 4). However, when either of the gases was applied at low temperature, the frequency was reduced 50% below that produced by X-rays in air at room temperature. The differences between low-temperature treatments and low-temperature plus gas treatments are significant at the 1% level with oxygen and at the 5% level with carbon dioxide (table 4).

TABLE 2

A comparison of the effects of X-raying (15,000 r) dormant seeds of barley in air, CO_2, and O_2 at room temperature (ca. 20° C) and at dry ice temperature (ca. −80° C), as measured by frequency of chromatinic bridges in root-tip cells of X_1 seeds and frequency of chromosomal interchanges at meiosis in X_1 spikes.

Treatment	Chromatinic bridges				Chromosomal interchanges			
	No. of expts.	Somatic cells obs.	Bridges observed		No. of expts.	Spikes obs.	Interchanges observed[1]	
			Total	Per cell			Total	Per spike
		No.	*No.*	*Av.*		*No.*	*No.*	*Av.*
X-rayed in air at room temp.	6	1113	275	.25	6	193	37.5	.19
X-rayed in air at dry ice temp.	4	560	111	.20	6	260	38.0	.15
X-rayed in CO_2 at room temp.	6	1086	374	.34	6	224	63.0	.28
X-rayed in CO_2 at dry ice temp.	4	441	63	.14	6	171	20.0	.12
X-rayed in O_2 at room temp.	4	368	113	.31	4	150	39.5	.26
X-rayed in O_2 at dry ice temp.	4	460	56	.12	4	201	27.5	.14
Not X-rayed, dry ice temp.	4	200	0	.00	6	206	.0	.00
Not X-rayed, CO_2 at room temp.	4	200	0	.00	5	125	.0	.00
Not X-rayed, O_2 at room temp.	4	200	0	.00	6	224	.0	.00
Control	4	200	2	.01	6	210	.0	.00

[1]Calculated on a "ring-of-four" basis. For example, a "ring-of-six" was considered as 1.5 "rings-of-four," involving a minimum number of breaks which could result in a "ring-of-six."

Frequencies of chromosomal interchanges in pollen-mother cells of X_1 plants. These data, as recorded in table 2, are summaries of at least four separate experiments for each treatment conducted in 1951 and 1952. Photomicrographs and descriptions of the types of chromosomal interchanges recorded can be found in CALDECOTT and SMITH (1952b).

The frequencies of the interchanges are increased (significant at the 5% level, table 4) approximately 40% by the presence of carbon dioxide and oxygen during X-radiation. However, the frequency is reduced by low temperature approximately 20% below that produced by X-rays at room temperature. Furthermore, the application of carbon dioxide or oxygen at low-temperature lowered the induced chromosomal interchange frequency by about 30% below that of air at room temperature. The differences between low-temperature treatments and low-temperature plus gas treatments are

TABLE 3

A comparison of the effects of X-raying (15,000 r) dormant seeds of barley in air, CO_2, and O_2 at room temperature (ca. 20° C) and at dry ice temperature (ca. −80° C), as measured by the frequency of seedling mutants in X_2 populations.

Treatment	No. of expts.	Head progenies tested	Mutations obtained	Heads with mutants
		No.	*No.*	*%*
X-rayed in air at room temp.	5	2365	162	6.8
X-rayed in air at dry ice temp.	5	3395	203	6.0
X-rayed in CO_2 at room temp.	5	2438	162	6.6
X-rayed in CO_2 at dry ice temp.	5	3001	203	6.7
X-rayed in O_2 at room temp.	4	2215	136	6.1
X-rayed in O_2 at dry ice temp.	4	2610	136	5.2
Not X-rayed, dry ice temp.	5	3798	3	0.08
Not X-rayed, CO_2 at room temp.	5	4552	6	0.1
Not X-rayed, O_2 at room temp.	4	1700	1	0.06
Control	5	1704	1	0.06

significant at the 5% level with carbon dioxide but not significant with oxygen (table 4). It is evident, then, that these results somewhat parallel the chromatinic bridge data.

Frequencies of seedling mutations among X_2 plants. The induced seedling mutation data presented in table 3 are summaries of at least four separate experiments for each treatment conducted in the greenhouse during the winters of 1951–52, 1952–53, and 1953–54. It is apparent that carbon dioxide or oxygen and low temperature either applied separately or combined had no statistically significant effect on the X-ray-induced mutation frequency (table 4). However, there is an indication that oxygen applied at low temperature reduced the mutation frequency.

DISCUSSION

The results reported herein demonstrate that low temperature (ca. −80°C) and/or a gas (carbon dioxide or oxygen) applied to dormant seeds during

X-irradiation modify certain of the X-ray effects in barley. Of particular interest are the following findings:

1. Radiation-induced injury as measured by seedling height and survival to maturity was reduced by low temperature but not affected by either of the gases.
2. Radiation-induced chromosomal aberration frequencies were increased by each of the gases applied at room temperature and were reduced by low temperature. The combination of either gas with low temperature resulted in a much greater reduction.
3. Radiation-induced mutation frequencies as measured by chlorophyll deficient seedlings among X_2 plants were not affected by either of the gases or by low temperature.

Under the conditions of this study, therefore, radiation-induced injury, chromosomal-aberration frequency, and mutation frequency were not affected to the same degree. This differential influence is readily discerned from an

TABLE 4

Tests of significance[1] of differences between meaningful comparisons for the five radiation effects. The tests are for X-rayed treatments and are based on the method outlined by DAVIES (1950).

Comparison	Seedling height	Survival to maturity	Chromatinic bridge freq.	Chromosomal interchange freq.	Mutation freq.
Air, R.T. *vs.* CO_2, R.T.	–	–	**	*	–
Air, R.T. *vs.* O_2, R.T.	–	–	**	*	–
CO_2, R.T. *vs.* O_2, R.T.	–	–	–	–	–
Air, R.T. *vs.* Air, −80°C	**	**	*	*	–
CO_2, R.T. *vs.* CO_2, −80°C	**	**	**	**	–
O_2, R.T. *vs.* O_2, −80°C	**	**	**	**	–
Air, −80°C *vs.* CO_2, −80°C	–	–	*	*	–
Air, −80°C *vs.* O_2, −80°C	–	–	**	–	–
CO_2, −80°C *vs.* O_2, −80°C	–	–	–	–	–

[1] ** = Significant at 1% level.
* = Significant at 5% level.
– = Not significant.

inspection of table 4. CALDECOTT and SMITH (1952a) have obtained somewhat similar results following heat treatments of dormant barley seeds before and after X-radiation. They reported that sub-lethal heat treatments either had no effect on, or reduced, the irradiation-induced injury, reduced the induced chromosomal-aberration frequency, but increased the induced mutation frequency.

The modification of induced injury by the low temperature and the failure of either low temperature or the gases to modify the induced mutation frequency agrees with earlier results where extremely low temperatures have been employed. STADLER (1931) reported such results following X-radiation of dormant barley seeds as they lay embedded in solid carbon dioxide (ca. −80°C). He attributed, as does the present author, the reduced injury to the

low temperature and not to the atmosphere. Subsequent papers (CALDECOTT and SMITH 1952a; NYBOM *et al.* 1953) have misinterpreted STADLER'S finding by claiming that he found marked reduction in mutation frequency following the low-temperature treatment. However, it is clear from the following quotation that no certain reduction in the mutation frequency was found (STADLER 1931). " Mutations were induced by radiation at a low temperature at a rate per *r* unit about ⅓ lower than that in the control. The difference in mutation rate was only about twice as great as its probable error, and it is therefore not improbable that mutability is unaffected even at this low temperature." KAPLAN (1951) has also reported a lack of modification of the induced mutation frequency following the X-radiation of dormant barley seeds at – 65°C. Radiation-induced sterility, the only other effect studied, was likewise not influenced by the low temperature. MAXWELL, KEMPTON and MOSLEY (1942) have reported a reduction in induced injury when dry maize seeds were held at temperatures from 0°C to – 187°C during exposure to X-rays.

The irradiation-induced chromosomal-aberration data pose several interesting points for discussion. The influence of carbon dioxide to increase the aberration frequency confirms the findings of SIZOVA (1936) in Crepis and of KING and SCHNEIDERMAN (1952) and SCHNEIDERMAN and KING (1953) in Tradescantia. Furthermore, they confirm the effect of oxygen on radiation-induced aberration frequency in several organisms as reported by many workers. Most of the pertinent reports on the oxygen effect have been reviewed recently by PATT (1953) and EHRENBERG *et al.* (1953).

Many reports of an effect of low temperature on radiation-induced chromosomal-aberration frequency have appeared in recent years (see reviews by CALDECOTT and SMITH 1952a; PATT 1953). Pertinent to this study are the results of FABERGÉ (1950, 1954) with Tradescantia and of NYBOM *et al.* (1953) with barley which demonstrated that extremely low temperature (liquid air, – 190°C) during the irradiation markedly reduced the chromosomal-aberration frequency. The data in the present paper support these findings. Furthermore, they demonstrate, possibly for the first time, that low temperature combined with either carbon dioxide or oxygen significantly reduces the irradiation-induced aberration frequency below that of low temperature alone. Apparently, the action of the gases is completely reversed at low temperature as compared with their action at room temperature. This suggests an interaction of gas and low temperature on the radiation-induced chromosomal-aberration frequency.

Possibly the most interesting and certainly the most important finding of this study is the apparent lack of correlation between radiation-induced chromosomal-aberration frequency and mutation frequency following the treatments applied. In this connection, NYBOM *et al.* (1953) have found a lack of correlation of X-ray effects under the influence of liquid-air temperature when dormant barley seeds were treated with 15,000 r. Compared with room temperature, the low temperature reduced the induced injury and chromosomal-aberration frequencies but increased the mutation frequencies. At 5,000 r and

10,000 r, however, low temperature reduced all three radiation effects. A theory of " intrasomatic selection " has been forwarded by NYBOM *et al.* (1953) and EHRENBERG *et al.* (1953) to account for the lack of correlation of X-ray effects at the higher dose. They maintain that an accumulative physiological killing of the most radio-sensitive cells occurs at higher doses which during the development of the plant eliminates the chromosomal aberrations and, to a greater degree, genetical changes. This elimination evidently was less marked when seeds were irradiated at liquid-air temperature. There are two points of evidence, however, that preclude the possibility of " intrasomatic selection " being a factor in the lack of correlation between the induced aberration and mutation frequencies in the present study: 1) At low temperature the mutation frequency was not affected even when the chromatinic bridge frequency in root-tip cells was reduced by 30 to 40 percent; 2) The mutation frequency was not affected when the meiotic chromosomal-interchange frequency was increased or decreased by the various gas and low temperature treatments—such chromosomal aberrations persist at least through meiosis I. Furthermore, CALDECOTT (unpublished) found from an X-ray dosage study, in which the same line of barley as in the present study was used, that the mutation and aberration frequencies were correlated at 15,000 r and slightly higher doses.

Evidently, the factors applied were affecting only the frequency of induced chromosomal aberrations and not the mutation frequency. Therefore, it follows that the induced seedling mutations were not correlated with at least the gross visible chromosomal aberrations, i.e., dicentric bridges and translocations. This view is compatible with the findings of YOUNG *et al.* (1953) who found in experiments using infrared treatments that recessive lethal and visible mutations do not result from chromosome breakage in *Drosophila melanogaster*.

Two hypotheses may be considered to explain the fact that the induced chromosomal aberration frequencies are altered while the mutation rate remains virtually constant, with the treatments used: (a) that chromosomal aberrations and " point mutations " are independent events, either of kind or of degree, and only the former are affected by the treatments accorded in the present study; (b) that the amount of ionization effect, resulting in both gross aberrations and " point mutations," is unchanged, but the amount of *reunion* of primary breaks is altered. In view of present knowledge, some of which is reviewed in the following paragraphs, hypothesis (b) seems the more likely—that the treatments do not alter primary breakage but affect reunion.

Evidence for the breakage hypothesis as compared with evidence for the reunion hypothesis has been reviewed and discussed by BAKER and EDINGTON (1952). The reunion hypothesis was favored by SCHWARTZ (1952) upon discovering that in maize there was a differential protection of low oxygen concentration against interstitial and terminal deletions. However, contradictory results recently have been obtained by KONZAK (personal communication). BAKER and VON HALLE (1953) arrived at the same conclusion as SCHWARTZ (1952) after finding the frequency of dominant lethals in mature sperm of *Drosophila melanogaster* induced by irradiation in nitrogen was reduced much

less than was expected on the hypothesis that oxygen concentration affects the number of primary breaks. SWANSON and SCHWARTZ (1953) have employed the reunion hypothesis to account for the differential effect of nitrogen on the frequencies of isochromatid and chromatid deletions in Tradescantia. More recently WOLFF (1954) has reported that the restitution of broken chromosome ends was enhanced by decreasing the oxygen tension in the cells during irradiation of Vicia seeds. A reducing agent, 2,3-dimercaptopropanol (BAL), was employed to decrease the oxygen tension. Furthermore, previous data (HAYDEN and SMITH 1949) from this laboratory which indicated a lack of correlation between induced mutation and aberration frequencies tend to support the reunion hypothesis. These data showed that X-raying (1200 r) germinating barley seeds in air at room temperature increased the chromosomal-aberration frequency 4.7 times, but increased the mutation frequency only 1.6 times the frequencies produced when the seeds were X-rayed in a vacuum.

CONCLUSIONS

From the data presented and the above discussion, it may be concluded that, at least in barley, X-ray-induced seedling mutation frequency—whether due to minute chromosomal rearrangements or intra-molecular changes—is not influenced by the presence of CO_2, O_2 or low temperature (ca. $-80°C$) during irradiation. These factors, however, strikingly alter the frequency of gross aberrations, both in root tips and in meiotic cells from X-rayed (dormant) seeds.

The differential aberration frequency can be visualized as arising from the action of temperature and the gases, or products of the irradiated gases, on the broken ends of the chromosomes. Low temperature in the presence of air, acts to facilitate restitution. This action is enhanced in the presence of carbon dioxide and oxygen. At room temperature carbon dioxide or oxygen, or substances produced by these gases under X-irradiation, react with the broken ends to reduce restitution.

The length of time during which the induced chromosomal breaks remain open in dormant barley seeds has not been ascertained. There is information at hand, however, that suggests the joining process may be delayed considerably, thus giving the gases and low temperature ample opportunity to act on the broken chromosome ends. KAPLAN (1951) has concluded that recombination movements in dormant seeds are hampered by the high viscosity of the cells. More recently ADAMS and NILAN (unpublished) have found an influence of atmosphere on induced chromatinic-bridge frequency up to six weeks after X-irradiation of dormant barley seeds.

That the effects of radiation can be modified differentially should be of considerable interest to those concerned with X-ray diagnosis and therapy. Such information is of immediate practical importance to the plant breeder, who is using ionizing radiations to produce viable and useful mutations. The data in this paper indicate that it is possible to maintain a radiation-induced mutation

frequency and at the same time reduce the induced chromosomal-aberration frequency and injury.

SUMMARY

X-raying dormant barley seeds at 15,000 r resulted in a virtually constant mutation rate whether the seeds were irradiated in air, oxygen, or carbon dioxide, at moderate (ca. 20°C) or low (ca. −80°C) temperatures. The rate of induced injury was reduced by the low temperature but not affected by the gases. The frequency of gross chromosomal aberrations was, however, strikingly altered by both low temperature and the gases. The lack of correlation between chromosomal-aberration and mutation frequencies was interpreted as showing that the gases and temperatures used were ineffectual with regard to primary X-ray damage, but profoundly affected the reunion of broken chromosome ends. The action of oxygen and carbon dioxide at room temperature increased the chromosomal translocation frequencies (decreased restitution). However, low temperature, alone or in combination with either of the gases, reduced the interchange frequencies (enhanced restitution). The increased X-ray tolerance of dormant seeds on solid carbon dioxide reported by TASCHER (1929) and STADLER (1931) was confirmed, as was their conclusion that the increased tolerance was due to the low temperature rather than to the presence of gaseous carbon dioxide.

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LITERATURE CITED

BAKER, W. K., and C. W. EDINGTON, 1952 The inductions of translocations and recessive lethals in Drosophila under various oxygen concentrations. Genetics **37**: 665–677.

BAKER, W. K., and ELIZABETH S. VON HALLE, 1953 The basis of the oxygen effect on X-radiated Drosophila sperm. Proc. Nat. Acad. Sci. **39**: 152–161.

CALDECOTT, R. S., and L. SMITH, 1952a The influence of heat treatments on the injury and cytogenetic effects of X-rays on barley. Genetics **37**: 136–157.

1952b A study of X-ray-induced chromosomal aberrations in barley. Cytologia **17**: 224–242.

DAVIES, V., 1950 Table showing significance of differences between percentages (for uncorrelated data). Wash. Agric. Exp. Stations Cir. No. 102; 24 pp.

EHRENBERG, L., A. GUSTAFSSON, U. LUNDQUIST and N. NYBOM, 1953 Irradiation effects, seed soaking and oxygen pressure in barley. Hereditas **39**: 493–504.

FABERGÉ, A. C., 1950 Chromosome breakage by X-rays at low temperature and the radiodecomposition of water. Genetics **35**: 104 (Abstract).
1954 Chromosome breakage by X-rays at very low temperatures. Radiation Research **1**: 130 (Abstract).

GILES, N. H., JR., 1950 Recent evidence on the mechanism of chromosome aberration production by ionizing radiations. Symposium on Radiobiology 267–284. John Wiley & Sons, Inc., New York.

HAYDEN, B., and L. SMITH, 1949 The relation of atmosphere to biological effects of X-rays. Genetics **34**: 26–43.

KAPLAN, R. W., 1951 Chromosomen- und Faktormutationsraten in Gerstenkörnern bei verschiedenartigen Quellungsbehandlungen oder Kälte während oder nach der Röntgenbestrahlung sowie bei Dosisfraktionierung. Z.i.A.V. **83**: 347–382.

KING, E. D., and H. A. SCHNEIDERMAN, 1952 The effects of carbon dioxide on the frequency of X-ray induced chromosome aberrations in Tradescantia. Proc. Nat. Acad. Sci **38**: 809–812.

MAXWELL, LOUIS R., J. H. KEMPTON, and V. M MOSLEY, 1942 Effect of temperature and time on the X-ray sensitivity of maize seeds. J. Wash. Acad. Sci. **32**: 18–24.

NYBOM, N., U. LUNDQUIST, A. GUSTAFSSON, and L. EHRENBERG, 1953 Biological effects of X-irradiation at low temperatures. Hereditas **39**: 446–457.

PATT, H. M., 1953 Protective mechanisms in ionizing radiation injury. Physiol. Rev. **33**: 35–76.

SCHNEIDERMAN, H. A., and E. D. KING, 1953 Further studies on the effects of carbon dioxide and oxygen on the frequency of X-ray induced chromosome aberrations in Tradescantia. Proc. Nat. Acad. Sci. **39**: 834–838.

SCHWARTZ, D., 1952 The effect of oxygen concentration on X-ray-induced chromosome breakage in maize. Proc. Nat. Acad. Sci. **38**: 490–494.

SIZOVA, M. A., 1936 Structural changes in chromosomes induced by irradiation of physiologically modified cells. C. R. Acad. Sci. U.R.S.S. **2**: 197–198.

STADLER, L. J., 1931 The experimental modification of heredity in crop plants. II. Induced mutation. Sci. Agric. **11**: 645–661.

SWANSON, C. P., and DREW SCHWARTZ, 1953 Effect of X-rays on chromatid aberrations in air and in nitrogen. Proc. Nat. Acad. Sci. **39**: 1241–1250.

TASCHER, W. R., 1929 Experiments with X-ray treatments on the seeds of certain crop plants. Ph.D. Thesis, Univ. Missouri. (Ms.)

WOLFF, S., 1954 Some aspects of the chemical protection against radiation damage to *Vicia faba* chromosomes. Genetics **39**: 356–363.

YOUNG, W. J., W. T. YOST, JR., P. T. IVES and R. PAUL LEVINE, 1953 The effect of pretreatment with infra-red radiation on the X-ray induced sex-linked recessive lethal and visible mutation rate in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **39**: 488–495.

ABSTRACTS OF PAPERS PRESENTED AT THE 1954 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

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ABRAHAM, S., I. H. HERSKOWITZ and H. J. MULLER, Indiana University, Bloomington, Ind. Genetic proof for half-translocations derived from irradiated oocytes of Drosophila melanogaster.* — Strong indirect evidence exists that X-ray-induced "detachments" of attached-X chromosomes in Drosophila oocytes containing no Y are usually due to rearrangements between the attached-X and an autosome. In such detachments, the attached-X is broken rather near its centromere, into a centric "J" and an acentric "I," and an autosome ("A") is broken subterminally (anywhere on 4 being subterminal). The stump of the J then becomes "capped" by the telomere-bearing autosomal tip, and/or the I becomes "captured" by attachment to the autosome's remainder in substitution for its tip. An egg receiving a capped J without I must, to survive, receive a complete maternal A, thereby becoming hyperploid for the autosomal tip, while one receiving a captured I without J is hypoploid for the tip. Either condition is genetically demonstrable if the tip includes a known marker. Capturing would also be recognizable by X-autosome linkage. — 39 detachment cases derived from oocytes irradiated 0-4 days before oviposition have been tested for hyperploidy with regard to markers near the ends of the autosomal linkage maps. 19 have so far proved to be half-translocations involving one or more markers. One involved capping by the tip of 2L, including gene al^+; two were cappings by 2R, both including $M22a^+$ and one sp^+ also; while 16 others were either cappings or capturings involving chromosome 4. This strong tendency toward X-4 translocations (compare independent finds of Lindsley and Novitski, D.I.S. 27) is most simply explained as resulting from the proximity of breakages (often heterochromatic) in appropriate positions in these chromosomes. (*Work supported by a grant from the U. S. Atomic Energy Commission (Contract AT(11-1)-195).)

ABRAHAMSON, S. and J. D. TELFER, Indiana University, Bloomington, Ind. Sex chromosome loss and translocation frequencies in Drosophila melanogaster after X-raying sperm in males or in females.* — Studies were undertaken of the effect of homogeneity, age, and stage of germ cells on X-ray mutagenesis. Sperm either in males (SM) or females (SF) were X-rayed and frequency of sex chromosome loss (partial or complete) and translocations determined by Muller's genetic stocks and procedures.

Females (Y^s . y InEN . Y^1; st), inseminated by 48-96 hr. old males (y In49 f B/sc^8 . Y; bw^D/bw^D) were given 600r and 3600r, respectively, while 48-60 hr. old sibling males were simultaneously given 3600r. One group of untreated females (SM1) was mated to treated males during first day after irradiation and another group (SM2) during second day. Losses involving either paternal sex chromosome were: control, 0.078±0.039% (4/5080); 600r SF, 0.35±0.05% (49-14,028); 3600r SF, 2.9±0.71% (16/550); 3600r SM1±SM2, 1.52±0.19% (19/1248). Translocation frequencies were: 600r SF, 1.43±0.24% (40/2795); 3600r SF, 26.2±2.78% (66/250); 3600r SM1, 11.0±2.78% (13/118); 3600r SM2, 4.8±1.26% (14/286). Both loss and translocation frequencies were lower from sperm treated in males than in females. (see similar results of Lüning in D.I.S. 27, which reached us after this work was under way). No difference was found for loss in first (1.42±0.64%, 5/343) and second day (1.55±0.41%, 14/905) inseminations, while the decline shown by the translocation frequency on second day is somewhat doubtful statistically. Mean losses of maternal X's, for eggs laid on first 12 days, were: control 0% (0/5330); 600r SF 0.236±0.04% (35/14,876) and 3600r SF 2.28±0.52% (18/787); later eggs of this period showed lower frequencies. Extent of participation of maternal chromosomes in the translocations is being investigated. (*Work has been supported by a grant to Dr. H. J. Muller and associates from the United States Atomic Energy Commission (Contract AT(11-1)-195).)

ALEXANDER, MARY L., FRANCES E. CLAYTON, and W. S. STONE, University of Texas, Austin, Texas. The induction of translocations by X-radiations at different stages of germ cell development in Drosophila virilis. — Induced translocations were used to demonstrate genetic damage to different stages of developing germ cells under several different physiological conditions. Males, 15 to 30 hours after eclosion, were X-radiated 2000 r in one minute at 0-5°C in a gas mixture with suitable pre- and post-treatment, mated individually to three marker females for 5 days; thereafter each male was remated every 48 hours for nine (A-I) mating periods. The first sperm used in inseminating females in lots A and B represent the advanced stages at irradiation; sperm used in subsequent matings were from earlier stages back to spermatogonia by H and I. In most tests the percent of translocation in B (7-9 days) corresponded to the values obtained for mature sperm treated under the same conditions. By D (11-13 days) or E (13-15 days) the frequency increased two or threefold. The values for air at the peak were 25 to 28% as compared to 17.2% for mature sperm from earlier experiments; in 96% N_2 + 4% O_2 the rate was 32% compared to 14%. In 95% CO + 5% O_2 there was a 39% peak at D and E, then an increase to 76% at F. After the peak, the rate drops to a value of 1% and less (spermatogonia). Early pupae produced a few offspring from meiotic or post meiotic stages with a translocation rate equivalent to that in the carbon monoxide mixture, then the rate fell to that of spermatogonia. (This work was supported by grants from the Rockefeller Foundation, and Atomic Energy Commission Contract AT-(40-1)-1323.)

ANNAN, MURVEL E., University of Nebraska, Linclon, Nebraska. Effects of X-rays on Drosophila robusta females. — Groups of ten virgin D. robusta females, 10 or 17 days old, were either exposed to X-rays (2,500 or 5,000 roentgen units) or served as untreated controls. Immediately after treatment each female was placed in a vial with two males. For 20 days after treatment, observations were made on fecundity and fertility. — The number of eggs laid was reduced by X-rays. Even though total egg production was reduced by 2,500 r, the reduction was less than 1/2 as great as in the 5,000 r treated series. — Dissection of the females on the 21st day showed the ovaries of those females which had been exposed to 5,000 r X-rays to be considerably atrophied. There were no detectable differences between the ovaries from females of different age groups treated alike or between the 2,500 r and control groups. — The reduction of egg-hatch by X-rays was nearly proportional to the dose for the first 10 days following treatment. From then on, variable recovery was exhibited. — A generally higher rate of X-ray induced dominant lethality was noted than that reported by Yanders (1954) in his study of D. robusta males. Whereas Yanders found a greater induction of dominant lethality in older flies compared to younger ones, there was no such effect of age when females served as the X-rayed parents.

ATWOOD, K. C., FRANK MUKAI, and THAD PITTENGER, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Neurospora techniques for large-scale studies of recessive lethal mutation. — The heterokaryon method for recessive lethals in Neurospora detects mutations leading to loss of essential functions anywhere in the genome. Special techniques have been developed for improving the speed and economy of the method, so that large-scale studies are no longer unduly laborious. These techniques involve a refinement of the sorbose plating method, rapid isolation of colonies from the plates by means of miniature open-ended culture tubes known as punch tubes, simultaneous sealing and supplying of additional medium to the punch tubes, and discharging the matured contents of each punch tube in proper dilution onto a portion of an agar plate. A Cornwall syringe and a special plate divider are used in this final step. Similar methods have been adapted to testing lethals of different origin for homology, hitherto almost prohibitively laborious. The steps in these procedures, use of the simple apparatus, and scoring of results will be demonstrated.

BAKER, WILLIAM K., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Chromosome association and segregation in polysomic Drosophila males. — By use of a translocated chromosome bearing the centromere and peach locus of chromosome 5 and all the fertility factors of the Y chromosome (designated 5^{Y}), a genetic study has been made of segregation in D. virilis males of the following constitutions: $XY5^{Y}$, $X5^{Y}5^{Y}$, and $XY5^{Y}5^{Y}$. The mode of segregation was determined by a system of progeny testing in which the 5^{Y} chromosome could be followed since it produces variegated eyes when the mutant allele of peach is pres-

ent. — The segregation data obtained from the $XY5^Y$ could be interpreted similarily to Kikkawa's conclusion
males, i.e., a bivalent and a univalent are formed and the bivalent forms more frequently between the Y chromosomes. However, on this basis the segregation results found with $X5^Y5^Y$ males are inexplicable since bivalents would have to be formed between an X and a 5^Y to almost the complete exclusion of bivalents between the two identical 5^Y chromosomes. This raised the suspicion that the chromosome association determining segregation was not of the bivalent-univalent type but rather a multivalent association. — A cytological study has been made of first meiotic metaphases in males of the three genetic constitutions listed previously. The results of this study justify the suspicion raised since a trivalent is formed almost without exception in the $XY5^Y$ and $X5^Y5^Y$ males and a quadrivalent in the $XY5^Y5^Y$ males. Photomicrographs of these multivalent associations will be shown.

BEATTY, ALVIN V. and JEANNE W. BEATTY, Emory University, Ga. The influence of oxygen on the physiological effects of X-radiation in the microspores of Tradescantia paludosa. — Previous results from studies on the effect of oxygen on the frequency of X-ray-induced chromosomal rearrangements in the microspores of T. paludosa showed, when X-radiation was carried out at 50 r per minute for 8 minutes, an aberration frequency of 24% in helium, 43% in 5% oxygen and 78% in air. The same type of experiment was carried out for testing the physiological effects of X-radiation using as criteria the unspiraling and reversion of early and mid-prophase stages, the retardation of each stage of division and the clumping of chromosomes. The primary effects were found to be similar to the secondary effects in that they were greatest in air, least in helium and intermediate in 5% oxygen. The greatest amount of reversion in air was recorded at the fourth hour following X-radiation, in helium at the two hour period and in 5% oxygen between 2 and 3 hours. The other categories of measurement exhibited a proportional effect in a similar manner.

BELL, A. EARL, Purdue University, Lafayette, Indiana. A gene in Drosophila melanogaster that produces all male progeny. — A full sib mating within an inbred line of Drosophila melanogaster gave more than 500 sons and no daughters. Subsequent matings revealed the genetic nature of this sex ratio abnormality to be a recessive gene, second chromosome, locus about 43. The name "daughterless" (symbol da) is suggested as best describing the gene. Females homozygous, da/da, have 100 per cent male progeny regardless of the genotype of their mates. Males homozygous, da/da, have normal sex ratios in their progenies when mated with females other than da/da. — This abnormal sex ratio appears to be due to a lethal action in the egg stage against the females not appearing. While similar to the gene reported by Helen Redfield (Genetics 11: 482-502), "daughterless" is more severe in its action and does not appear to allelic. — The disguise of Sturtevant's "transformer" gene, which transforms females into males, was inadequate to escape the lethality of "daughterless." No XX tra/tra

individuals (females transformed into males) appeared among the 100 per cent male progeny from da/da females.

BLAIR, W. FRANK and DAVID PETTUS, University of Texas, Austin, Texas. Differentiation in mating call among southwestern anuran amphibians. — Differences in mating calls are important mechanisms of reproductive isolation in anuran amphibians, but until recently no objective method of comparing calls has been available. The calls of representative southwestern anurans have been analyzed with a "Sona-Graph," which gives a visual representation of the call in respect to time, frequency and intensity. A few basic patterns of calls have been so modified that the call of each species of anuran studied differs markedly from that of every other. Species within the same genus are distinguished by variations in at least one major attribute of the call. Variations in call occur within local populations; geographic variation in call occurs in widely distributed species. (This work supported under National Science Foundation Research Project NSF-G328.)

BLIGHT, WILLIAM C. (Introduced by H. L. Carson.), Washington University, St. Louis, Mo. A study of population structure in Drosophila americana near St. Louis, Mo. — Populations of this species appear to be limited to areas immediately adjacent to inland waters. The species is polymorphic for two alternative gene arrangements on each of three chromosome arms. These alternatives are designated Xab, Xabc; 4, 4ab; 5a and 5b. Population samples have been taken from various stations along the Meramec River in 1952 and 1953. Collections made in June, July and August are termed summer samples; those made in September, October and November are referred to as fall samples. — The fall '52 sample from the Highway 21 station showed a significant increase in the frequency of the 5b gene arrangement compared with summer '52. Fall '52 samples from Bauer Road, 3.3 miles upstream, and Eureka, 20 miles upstream, were also analyzed. Inversion frequencies in the Highway 21 fall and Bauer Road populations differed by less than 1% suggesting that the two samples were taken from a single large population. A comparison of Highway 21 fall and Eureka indicated a significant difference in frequency of the Xab and 5b arrangements at the two stations. — The summer '53 sample from Highway 21 showed significant shifts in the frequencies of the Xab, 4ab and 5b as compared to the previous fall. The frequency of Xab, having increased 14% from fall '52 to summer '53, declined to the previous level in the fall '53 sample from the same location. A fall '53 sample was taken from Eureka and a new station 147 miles upstream from Highway 21. There were no significant differences in the inversion frequencies at any of the three stations. — It appears that overwintering populations are subjected to genetic drift, whereas the relative stability of the larger fall populations is due to selection pressure.

BOWDEN, WRAY M., Department of Agriculture, Ottawa, Canada. Cytotaxonomic and genetic studies in Section Dortmanna of the genus Lo-

belia. — The North American species have been evolved from diploid ancestral populations. Five distinct lines of speciation can be traced. In each of three lines, only a single diploid (2n = 14) species has survived: L. inflata, L. kalmii and L. dortmanna. The fourth line consists of small-flowered species: L. nuttallii, L. feayana, L. canbyi, L. boykinii, L. appendiculata and L. spicata; the last diploid species is composed of four intergrading varieties. The fifth line has evolved the large-flowered species of this section; evolutionary centres were (1) the coastal plain and (2) the Appalachian Mountains. In both areas are found two old diploid species, L. puberula and L. georgiana. L. brevifolia is a primitive diploid coastal plain species and shows relationship to both L. puberula var. puberula and L. georgiana. L. reverchonii is a diploid coastal plain species derived from L. puberula and probably L. siphilitica var. ludoviciana. There are three amphidiploid (2n = 28) species: (1) L. elongata and (2) L. glandulosa were derived in the coastal plain from hybridization of L. puberula var. puberula, L. georgiana and perhaps L. flaccidifolia; and (3) in the Appalachian Mountains, L. amoena was derived from L. puberula var. simulans and L. georgiana. The mountain species, L. puberula var. simulans, is the probable ancestor of L. siphilitica and L. cardinalis has been derived from the latter. The evolutionary development of the large-flowered species has culminated in L. cardinalis subsp. cardinalis and subsp. graminea.

BOWEN, C. C.* and A. H. SPARROW, Brookhaven National Laboratory, Upton, New York. Radiosensitivity of several meiotic stages of Lilium** — The allometric relationship of mean bud length to stage of microsporogenesis in Lilium longiflorum var. Croft has been worked out in detail. This information has permitted the x-irradiation of anthers at known meiotic stages in intact buds. Cells were fixed at first and second meiotic anaphase and at first microspore division. Analysis of this material has shown a wide variation in the radiosensitivity of the several different stages. Late meiotic prophase showed extreme sensitivity compared to other stages as has previously been shown in Trillium. The high frequency of first anaphase bridges without fragments following irradiation at very late prophase and first metaphase will be discussed in the light of Crouse's work (Science 119: 485-487, 1954) on half-chromatid breaks, which these data appear to confirm. (*Public Health Service Research Fellow of the National Cancer Institute,) (**Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

BLAUCH, BERTINA M., University of Pennsylvania, Philadelphia, Pa. Dzierzon's law and free oviposition in Melittobia. — According to Dzierzon's law eggs of the honey bee are all potentially male-producing if unfertilized, female-producing if fertilized. In the chalcidoid wasp Melittobia mated females lay many eggs almost all of which are fertilized and develop into females, but a few remaining unfertilized produce males. Unmated females normally lay very few eggs and these, if they develop, produce males. It has been suggested that their unlaid eggs are different from

their laid eggs in that they are potentially female-producing and incapable of development unless fertilized. In a selected stock of M. species-C many unmated females are free egg layers, lacking the normal block to oviposition. Although all of their offspring are males, it has not hitherto been shown that female-producing eggs, infertile unless fertilized, are lacking. Three free egg layers were isolated on host larvae, Sceliphron, and their eggs were counted every day or two until their death after eight days. From the 385 eggs counted (a very few may have been missed) there developed 378 males. It may therefore be concluded that all eggs are capable of development and are male-producing without fertilization.

BOYES, J. W., McGill University, Montreal, Canada. Karyotypes and their measurement in higher Diptera. — Chromosome complements from the brains of species in families such as the Anthomyidae, Calliphoridae, Sarcophagidae, and Tachinidae have been drawn, measured and photographed. Idiograms of these somatic complements constitute a basis for comparison of species, genera and families. Most species have 12 chromosomes but numbers range from eight to 19 or 20. There are extensive variations in chromosome morphology both within and between families. Sex chromosomes are more variable in morphology than the autosomes in most families and both X : Y and X_1X_2 : Y sex-chromosome mechanisms have been found. Several examples of individual flies deficient for one sex chromosome and of the presence of supernumerary chromosomes of various types have been seen. These studies indicate that higher Diptera have greater stability in both chromosome number and morphology than has been found in Drosophila species and emphasize that precise methods reveal variations between very similar complements which with ordinary examination would be erroneously considered as the same.

BRILES, W. E., Texas A. and M. College System, College Station. Evidence for overdominance of the B blood group alleles in the chicken. — Three White Leghorn inbred lines having a common origin have been examined for two seasons for possible effects of the B blood group alleles on hatchability, body weight at nine weeks of age, and egg production. Chicks from each of the lines were tested at four weeks of age with blood typing reagents prepared from iso-antisera made within the lines. Because of the several genotypes resulting from the three B alleles present in each line, all birds were classified as either heterozygous or homozygous. In collecting hatchability data matings were grouped according to the percent heterozygosity expected among the zygotes based on the blood types of the parents. The hatchability of fertile eggs produced by matings producing 0, 50, 75, and 100 percent heterozygosity were 46, 62, 71, and 78 percent, respectively. These data indicate that the probability of heterozygous embryos hatching is about 1.7 times that of homozygous embryos. — In two of the three inbred lines under study (lines 22 and 23) the body weight at nine weeks of age averaged 5 to 10 percent greater for the heterozygous than for the homozygous chicks. The third inbred population (line 24) showed no effect of the heterozygous condition at the B locus on growth in either

of the two years. This differential effect on growth suggests that the genetic background afforded by the different lines plays a significant role in determining whether or not the heterozygosity at the B locus will affect growth rate. — The average monthly egg production for the three inbred lines, where samples of twelve or more birds of each genotypic class were available for comparison, favors the heterozygous females. The average egg production of the heterozygous classes within each line and season was from nine to thirty percent greater than for the homozygotes. — Heterotic effects on such widely different characteristics as hatchability, growth rate, and egg production not only indicate that the phenomena of overdominance is operative but suggest that the condition of heterozygosity at the B locus is influencing rather strongly one or more fundamental physiological processes the effects of which are distributed throughout the life cycle of the organism. (This investigation was supported in part by a research grant (G-3332) from the National Institutes of Health, Public Health Service.)

BRAUN, WERNER, JEANNE WHALLON, and W. L. MAUZY, Camp Detrick, Frederick, Md. Further data on the selective effects of DNA upon bacterial population changes. — In studies with Brucella strains two different effects of DNA upon population changes have been observed: (1) transformation-like phenomena which occur when competent strains are exposed to highly polymerized heterologous DNA, and (2) a non-specific selective effect, which involves an inhibition of growth of the parent type cells while favoring continued growth and the rapid establishment of mutant type cells. The latter effect can be observed with certain strains following the addition of any bacterial DNA so far tested after it has been briefly exposed to DNase (Braun and Whallon, PNAS, 40: 162, 1954). The intensity of the selective effect depends greatly upon the production of a compound produced by mutant cells in the presence of enzyme-treated DNA. Some information on the general nature of this compound has been obtained in studies on the selective effects of variously treated filtrates obtained from mutant cultures containing DNA + DNase. In certain media these selective effects also occur to some extent when cultures are supplemented with only DNA or only DNase. This suggested that, depending on the medium employed, the cells themselves may produce both DNA and DNase. This was verified by chemical tests and it was shown that the medium-dependent selective effects are not correlated with differences in the amount of DNA accumulating in different media but appear correlated with medium-dependent differences in the ability of DNase to act upon DNA. The implications of these observations for a general consideration of the effects of nucleic acids upon the selective establishment of different types of cells will be briefly discussed.

BROWN, META S., Texas Agricultural Experiment Station, College Station, Texas. A comparison of pachytene and metaphase pairing in species hybrids of Gossypium. — Study of pachytene pairing in species hybrids of Gossypium has led to the following observations and conclusions: Chro-

mosomes of differentiated genomes pair intimately at pachytene, independently of the degree of metaphase association. Pachytene pairing in sterile as well as fertile hybrids appears as intimate as within species. Pachytene pairing in hybrids cannot be considered a measure of chromosome or species differentiation, and metaphase pairing remains the best criterion of chromosome homology as now defined. The extent to which non-homology is due to structural differences in Gossypium cannot be determined by pachytene studies. Not chromosome pairing, but chiasma formation appears to be the critical event for metaphase association in species hybrids. Paired pachytene chromosomes of different genome groups are equal in length despite differential size at metaphase. The maintenance of metaphase chromosome size characteristic of a species in chromosomes isolated from the parent species, and independent of pachytene length, suggests a mechanism controlling size which is autonomous within individual chromosomes. Reasons are given why neither structural differences nor genic action can account for all the pairing behavior of Gossypium chromosomes, and the question is raised whether a mechanism analogous to that controlling size may not control chiasma formation in Gossypium.

BURDICK, A. B., Purdue University, Lafayette, Indiana. Two types of heterosis in the tomato revealed by constant parent regression analysis. — Heterosis is manifest in the tomato in increased size of plant and in increased sexual earliness. In crosses of widely different types of tomato, the "average" hybrid plant is 28% heavier than its largest parent at 90 days of age. Such hybrids are usually intermediate with respect to their parents in time of flowering but will produce ripe fruit 1.62 days earlier than their earliest parent. — Constant parent regression analysis of plant size and earliness data give $b_2 = 0.0010$ for plant size and $b_2 = -0.0333$ for earliness (period from flowering to ripe fruit). Genetic interpretation would allow dominance as a cause of the negative b_2 for earliness but, by the same inference, would not allow dominance as the cause of the positive b_2 for plant size. Sufficient reason exists to attribute the positive b_2 to epistasis. — Earliness data may be further analysed to show distinctly different patterns of manifestation of heterosis in different hybrids. These patterns indicate that the genes of one parent may control development at one time while the genes of the other parent may do so at another stage. The term co-dominance is proposed to describe this phenomenon which may partially account for the general excellence of hybrids.

BUTLER, L., University of Toronto, Toronto, Ontario. The relation of squint, eyes open at birth, and wavy in the house mouse. — The eye abnormality squint is caused by wide alterations in the rates of development of the various parts of the eye. Squint mice often have their eyes open at birth and since the gene "o" has been postulated for this phenotype the independence or association of o & sq had to be established. Both "eyes open" and squint occur independently but breeding data indicate that such occurences are the result of differences in expressivity of a single gene instead of segregation of two genes. The close association of squint with

wavy distinguishes it from o but also suggests the hypothesis that squint is a pleiotropic effect of wa-2. The occurence of 3 Wa sq in an F_2 of 285 mice, and the limited breeding results from these, indicate that "squint" is a separate gene with incomplete penetrance. In the wa-2 sq line the penetrance has increased from 80% to 94%, while the wa-2 Sq line breeds true.

CLAYTON, FRANCES E., University of Texas, Austin, Texas. The development of the compound eyes of lozenge alleles in Drosophila melanogaster. — The development of the compound eyes of normal D. melanogaster and nine lozenge mutants was studied. Analysis of the larval and pupal differentiation indicates that the lozenge anomalies begin to develop at the end of the first day of pupal development. Those lozenge mutants which possess some normal facets in the adult eyes (lz, lz^{BS}, lz^{g} and lz^{34}) are similar in their development, differing only in the severity of the abnormalities which develop. These mutants are characterized by the presence of a layer of abnormal retinulae located in the postretinal region of the eye. The lozenge mutants which lack normal facets in the adult eyes (lz^{3}, lz^{y4}, lz^{s}, lz^{sB} and lz^{36}) develop similarly, being characterized by the failure of the retinulae to penetrate the outer layer of pigment cells ar ' the failure of the primary pigment cells and pseudocone cells to differentiate normally. This results in the formation of a layer of pigment cells immediately below the cornea and in the distortion of the retinulae below the layer of cornagen cells. One of the basic mechanisms involved in the production of the lozenge type eye appears to be the failure of the ommatidial cells to differentiate normally during pupal development.

DERMEN, HAIG, U.S. Plant Industry, Beltsville, Maryland. Histogenetic factors in color and nectarine sports of peach. — a) A white colored somatic mutation, a rare instance of a change from a recessive to a dominant gene, has occurred in yellow fleshed Elberta variety. At fruit suture there is a yellow line bordered by white flesh. Some fruits are white except for a yellow suture. Most fruits have white flesh under the skin but yellow in varying patterns by the pit. b) Nectarine sport occurred somatically in J. H. Hale variety. On trees propagated from the sport branch most fruits are normal, hairy skinned; a few fruits are fuzzless like nectarine; many fruits are part fuzzy and part fuzzless. Nectarine character is ordinarily controlled by a recessive gene. The mutation described here behaves as if it were a dominant one. Variations in color, and fuzzy and fuzzless patterns in the two sports are shown to have a histogenetic basis. It is concluded that the color mutation has occurred in L-II in the shoot apex, and nectarine mutation in L-III. Diffusion of some material from tissue developed from L-III may be affecting hair development in the epidermis developed from L-I.

DERMEN, HAIG, U.S. Plant Industry, Beltsville, Maryland. Location of cells and mode of mitosis. — Cells at the shoot apex of angiosperms are arranged in layers. This is because cells in L-I almost invariably

divide anticlinally, in L-II anticlinally rather generally but with some periclinal division occurring, and in L-III and deeper in apex the mode of division is random. Epidermal tissue in peach fruit is one cell thick and is derived from L-I. In the development of ovary two edges of a U shape carpel meet. The suture in peach fruit and other fruits of the genus Prunus corresponds to the line where edges of the carpel have met. Therefore, in the suture area there are originally two layers of cells derived from L-I. In studying cytohistologically 4-2-2 or 2-4-4 types of cytochimeral peaches it was generally found that the tissue at the suture originating from L-I was more than two cell-layers thick. This is taken as evidence that mode of mitosis is influenced by the position of cells in parts of the plant as in the shoot apex and in the ovary. Certain forces, such as protoplasmic movement, may be affecting the orientation of mitotic spindle depending on the location of cells.

DOUDNEY, C. O., Biochemical Genetics Laboratory, Department of Zoology, University of Texas, Austin, Texas. Gene interaction and temperature response of the threonine inhibited strain of Neurospora. — Studies of the reversal of threonine inhibition of a strain of Neurospora crassa (UT77a) indicated that threonine inhibits growth by interfering competitively with the metabolism of homocysteine, thereby interrupting the biosynthesis of methionine, the thiazole moiety of thiamin, and, at higher concentrations, adenine and serine. In order to determine the effect of genetic interruption of threonine biosynthesis on the threonine inhibition process, double mutant strains possessing the gene mutation responsible for threonine inhibition and a gene mutation causing an absolute threonine requirement were constructed. One such strain (UT77, 44104) gave growth responses to threonine equivalent to the parent threonine requiring strain at concentrations of threonine effecting complete inhibition of growth of UT77. In this case the 44104 gene mutation suppresses the UT77 phenotype. However, another such strain (UT77, 35423) was even more markedly inhibited by threonine than UT77. Incubation temperature studies revealed that at lower temperatures UT77a requires either the thiazole moiety of thiamin or both homocysteine and threonine. The basis for the double amino acid requirement is a competitive antagonism between threonine and homocysteine, the presence of either in the medium producing a requirement for the other. The thiazole requirement is probably due to an interference of endogenously formed threonine with homocysteine metabolism. These facts suggest that the phenomena of growth inhibition by competitive mechanisms at the metabolic level may be one basis for temperature sensitive genetic blocks. Furthermore they suggest that a gene mutation which reduces biosynthesis of the inhibitory metabolite may suppress such a genetic block to biosynthesis. — These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Texas, NR 859(00).

DUNCAN, ROBERT E., and JOHN W. WOODARD, and PHILIP S. WOODS,* University of Wisconsin, Madison, Wisconsin. Cytological effects

of sodium barbital. — Immersion in aqueous solutions of sodium 5-5 diethylbarbiturate (sodium barbital) 2500 p.p.m. completely inhibits root growth of Alium cepa and Vicia faba. At 1250 p.p.m. roots grow about half as rapidly as in distilled water. Dependent upon the extent of further dilutions sodium barbital may be less inhibitory, ineffective, or even stimulatory. Decreased growth at 1250 p.p.m. in Vicia, accompanies a decrease in the mitotic index. The proportion of nuclei at prophase to those at metaphase is greater in treated than untreated meristems. Among nuclei at prophase are many with shortened chromosomes and conspicuous polar caps, transition from prophase to metaphase apparently being delayed. The return to normal during recovery is rapid. According to photometric evaluation of the content of Feulgen stain (DNA equivalent) interphase nuclei in onion root meristems treated with 1250 p.p.m. belong to 2C, 4C, and intermediate classes in the same proportion as in untreated meristems. Some system other than synthesis of DNA must be affected and causes the decrease in mitotic index. Interference in this system apparently is responsible for the changes in prophase-metaphase ratio and the delay in transition from prophase to metaphase. (*Now at Dept. of Zoology, Columbia University, New York, N. Y.)

FABERGÉ, A. C., University of Missouri, Columbia, Missouri. The analysis of chromosome breaks by endosperm phenotype in maize.* — The procedure consists in irradiating pollen carrying the dominant endosperm markers I, Sh, Bz, Wx, in the short arm of chromosome 9, and using the corresponding recessive as the female parent. Enough data have now been accumulated to permit a general evaluation of the method which, in some respects, gives information not readily obtainable by other means. A complete balance sheet of the fate of chromosome breaks is complicated, but can, as a first approximation be summarized as follows: A broken end may (1) restitute; (2) rejoin with another free end to give an aberration; (3) remain free and give a chromatid breakage-fusion-bridge cycle. An estimate of the minimum proportion of breaks restituting is roughly 9/10 of all breaks, and may well be larger. Free ends produce a breakage-fusion-bridge cycle, and do not in general become stable; apparent stable terminal losses being usually interstitial deficiencies. The point of origin of a breakage-fusion-bridge cycle being a single break, from the frequency of such breaks arising in marked chromosome segments, a map may be constructed. This chromosome map is not very different from the standard linkage map; there is some deformation, the region distal to I, and the region between Wx and the centromere having an excess of breaks relative to their genetic lengths. (*Work supported by a research grant from the National Cancer Institute, National Institute of Health, Public Health Service.)

FORRO, F. (Introduced by R. S. Caldecott), Brookhaven National Laboratory, Upton, New York. P^{32} distribution among the progeny of labeled bacteria*. — It is possible that the stability of genetic mechanisms indicated by linkage group and mutation data may have as an underlying basis

the chemical stability of the molecular species involved. One component considered to have genetic function is desoxyribose nucleic acid. Chemical studies do not yet present a clear picture of the metabolic stability of this molecule. By determining the distribution of a radioisotope among the progeny of labeled organisms, one might hope to trace the presence of stable genetic molecules which would follow a genetic segregation pattern. — Micrococcus cryophilus, chosen because it is characterized by a small number of "nuclear bodies" per cell and non-clumping growth habit, was grown in medium containing P^{32}, washed free of P^{32}, and regrown in non-labeled medium. Radioautographs were made on smears of the organisms at various times during the growth. The distribution of the P^{32} in the population was determined by microscopic grain counting of the autographs of individual organisms. Over the range of grain densities used, the average grain count per organism is a linear function of the decay-corrected exposure and of the P^{32} content per organism. Cytochemical techniques were used to define the phosphorus fractions under examination. The results indicate a randomization of the DNA phosphorus among the progeny to the extent that a model is required which is based on partition of the DNA into more than four segregative and chemically stable units per organism. (*Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

FORSTHOEFEL, PAULINUS F., University of Detroit, Detroit, Mich. Further studies on the developmental genetics of luxoid, a skeletal variation in the house mouse. — A previous report described an average caudad shift of the hind limb field of one segmental length in $10\frac{1}{2}$ day lu/lu mice. A restudy of the material using a more refined criterion has shown no significant difference between the sample in which luxoid was segregating (mean: 25.486 segmental units) and the sample of normal embryos (mean: 25.459 segmental units). Since luxoid has as one of its effects an increase in the number of presacral vertebrae, there probably is a real caudad shift of the hind limb field. The failure to detect the shift with certainty can be explained by another effect of the luxoid gene postulated to explain the forelimb polydactyly of lu/lu mice, viz. a caudad shift of the competent tissue for the forelimbs. In the study of the possible caudad shift of the hind limb field, it was assumed that the intersegmental artery associated with the subclavian artery was a fixed anterior point in both lu/lu and normal embryos which could serve as a reference point in measuring the extent of a caudad shift of the hind limb field. Since the subclavian artery is intimately associated with the forelimb bud, it is probable that a caudad shift of the forelimb field in lu/lu embryos causes a caudad shift of the subclavian artery and associated intersegmental artery, and prevents detection of the caudad shift of the hind limb field by the method used. This hypothesis of a caudad shift of the subclavian artery will be tested.

FOX, ALLEN S., Michigan State College, East Lansing, Michigan. Paper chromatographic studies of the effects of the lozenge pseudoalleles and the Y-chromosome in Drosophila melanogaster. — Antigenic effects of

the lozenge pseudoalleles have been previously demonstrated (Chovnick and Fox, 1953, Proc. Nat. Acad. Sci. 39: 1035). The possibility of similar effects on protein precursors (amino acids and peptids) has now been investigated by means of paper chromatography. One-dimensional and two-dimensional chromatography of beheaded flies, squashed on Whatman No. 1 paper, with 80% aqueous phenol as first solvent and butanol-acetic acid-water (4:1:1) as second, affords satisfactory separation of ninhydrin-positive materials. The following amino acids have been tentatively identified in both sexes: aspartic, glutamic, serine, cystine, ornithine or taurine, glycine, lysine, threonine, glutamine, alanine, arginine, tyrosine, histidine or citrulline, valine, norvaline, methionine, tryptophane, and the leucines. Four additional ninhydrin-positive substances, probably peptids, are found in both sexes. Examination of all homozygous and heterozygous lozenge genotypes and coisogenic wild discloses no qualitative or quantitative differences. It is concluded that these genes produce their antigenic effects late in protein synthesis. — Males possess a ninhydrid-positive substance, probably peptid, not present in females. The sexes also differ with respect to a number of unidentified ultraviolet flourescent and absorbing substances, separated with propanol-ammonia (2:1) as first solvent and butanol-acetic acid water (4:1:5) as second. In a series of coisogenic stocks, males with and without a Y, or with an extra Y^S or Y^L, all exhibit the typical male patterns. Females with or without a Y, or with a Y^S or Y^L or both, all exhibit the typical female patterns. The heterchromatic Y-chromosome is therefore not responsible for the chromatographic differences between the sexes. — Supported by a grant from the National Institutes of Health.

FUERST, ROBERT., Genetics Laboratory, Department of Zoology, University of Texas, Austin, Texas. Differences in free intracellular amino acids in Neurospora. — The growth of Neurospora results in the accumulation of metabolic products in the medium, and in the production of free intracellular substances, such as amino acids, inside the cell. A correlation between the composition of the internal free intracellular nitrogen pool with protein synthesis was attempted. — By the use of chromatographic methods some 31 ninhydrin-sensitive substances were encountered in cell extracts containing free intracellular substances. — Qualitative and quantitative differences in amino acids were found to be associated with certain mutant characteristics. In some of the mutants certain amino acids were consistantly absent from the free amino acid pool. Other apparent differences between mutant and wild type disappeared in backcrosses to wild type, and new amino acids showed up. — The differences in the amino acid patterns are quite complex, but sufficient evidence is now available by the experiments to be described to assert that differences in free amino acids exist in certain Neurospora mutants. — These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Texas, NR 859(00).

FUNG, SUI-TONG CHAN and JOHN W. GOWEN, Iowa State College, Ames, Iowa. Histological observations on the gonads of Drosophila melano-

gaster heterozygous for the hermaphroditic gene, Hr. — In early second-instar larval stage, the inter-mediate size of the hermaphroditic gonads is clearly differentiated from that of the normal males and females. The normal ovary contains small, compact cells with no definite arrangement. The testis is large and the gonial cells are uniform in size. The hermaphroditic gonad has two types of cells: small oogonial-like cells located at the two ends of the organ and larger cells, usually in a cluster of 8 or 16 found in the central portion of the gonad. These larger cells have a characteristic cytological appearance. Their nuclei are large, deeply-stained, and seem to contain chromosomes in polytene condition. They are recognizable to the late pupal stage. Their significance to sexual development is not clear. In the adult the hermaphroditic gonads are generally rudimentary but in some instances may attain mature size with irregularly arranged follicles. The hermaphroditic gonad lacks the thin, nucleated epithelium sheath that encloses the egg chambers in the normal ovariole. As a result, all egg follicles are in the form of loose egg-strings or enclosed in the peritoneal sheath. Yellow pigmented tissue characteristic of the testis sheath in normal males may associate with the hermaphroditic gonads. When isolated and studied histologically it shows that the cells resemble vasa efferentia. Three distinct cell layers are present. The external epithelium has large flat cells with bulging nuclei. The thin inner sheath has small cells. The thick innermost layer is formed of columnar cells. The presence of the external layer, with large nuclei and bulging cells, and the yellow pigmentation in the vasa efferentia even though the normal testis is absent, indicates that the hermaphroditic gonad, although potentially XX, can develop the pigments characteristic of the normal male.

GARBER, E. D., The University of Chicago, Chicago, Ill. The orientation of multivalents at metaphase I in the subgenera Para-Sorghum and Stiposorghum, genus Sorghum. — The subgenera Para-Sorghum and Stiposorghum, genus Sorghum (x = 5), include diploid and tetraploid species. Two of the 3 tetraploid species in Para-Sorghum have 1-5 ring quadrivalents at diakinesis and MI. In one species, S. leiocladum, open and zigzag rings occur with approximately equal frequencies at MI; in the other species, S. australiense, most of the rings are zigzag at the same stage. Sorghum plumosum (Stiposorghum) includes both tetraploid and hexaploid types. The tetraploid has 1-5 ring quadrivalents at diakinesis and MI; the hexaploid has mostly bivalents, quadrivalents, and hexivalents at the same stages. Most of the multivalents in this species are zigzag at MI. The directed orientation of multivalents at MI is considered to be genetically determined in S. australiense and S. plumosum but not in S. leiocladum. The implications of this interpretation will be discussed. (Supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.)

GOWEN, JOHN W. and JANICE STADLER, Iowa State College, Ames, Iowa. Effect of acute and chronic X-ray and nuclear irradiations on life

spans of different strains of mice. — Four effects of irradiation from a high energy source concern those exposed; acute sickness and death following immediately on irradiation, prolonged but less acute effects resulting in impaired mental or physical health over the rest of the life span, changes in expected life span, and possible progeny effects as in sterility, abnormal mental or physical development, or unfavorable inheritance. Mice from 12 of our different inbred strains have been exposed to X- and gamma rays and neutrons from nuclear detonations. These animals are mated in pairs. Data are taken on the general health, behavior, fertility, mothering ability, and life span of exposed animals. Their progeny are observed to four weeks old for the same characteristics, and at necropsy for any internal lesions. Data on life expectancy, covering the four year period that the experiments have been in operation, may be interpreted as follows. Immediately following irradiation direct damage is expressed by increasing numbers of deaths as irradiation exposures become greater. The increases in deaths result in sharp reductions in expected life spans of exposed individuals. For single exposures the reductions in life expectancies are slightly curvilinear when plotted against dose. Where the irradiation doses are divided in two or five exposures with weekly intervals between, the reductions in life expectancies are linear with dose. Male and female mice show similar reductions in life span when exposed to like roentgen doses. Life expectancies for mice at exposure are reduced 0.31 days per roentgen where the irradiation is received in one dose, 0.29 days per roentgen where the dose is received during 2 exposures, and 0.15 days when exposures are 5 in number. Seventy-five to 100 days post irradiation covers the period of acute radiation effects. Following this critical period, life expectancy increases sharply. At 200 days the life expectancies of mice receiving the same total dose are reduced by 0.13 days for single exposure mice, 0.14 days for two treatments, and 0.11 days for 5 treatment mice. Life span differences are expressed at other periods and as affected by inheritance will be discussed.

HERSKOWITZ, I. H. and A. SCHALET, Indiana University, Bloomington, Ind. Sex-linked recessive lethal mutations connected with gross chromosomal rearrangements following nitrogen mustard treatment of mature Drosophila sperm.* — The number of recessive lethals associated with gross rearrangements has been found repeatedly by others to be fewer after chemical than after X-ray treatment. However, previous work with chemical mutagens may have included tests of gametes which at the time of treatment were not mature, and did not show the same relation between chromosomal breakage and/or union as obtains for mature sperm (which were more likely to be tested in the X-ray studies). It therefore seemed desirable to test this relationship by means of nitrogen mustard treatment of sperm known to be mature at the time of exposure. Accordingly, sex-linked recessive lethals in Oregon-R sperm, induced at the rate of 4.3% following sperm baths and 6.9% after vaginal douches with 1/4-2% methyl bis (beta-chloroethyl) amine hydrochloride, were tested for the simultaneous presence of gross rearrangements involving the lethal X. A gross

chromosomal rearrangement was detected by the reduction in crossing-over by 50% or more in either or both regions marked in heterozygous females carrying a lethal X and one including the recessive factors y v car. Lethal and rearrangement were considered associated wherever the rearrangement reduced crossing-over in one region of the chromosome in which the lethal was also present, or when crossing-over was reduced in both regions tested. Only 4 of 51 lethals of separate origin were associated with gross rearrangements, or about 8%, whereas at least 12% would be expected following X-radiation. — These data are in agreement with the earlier ones. Various explanations for this relationship will be discussed. (*This work has been supported by a grant received for work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

HEXTER, W. M., Amherst College, Amherst, Mass. A population analysis of heterozygote frequencies in Drosophila melanogaster. — By means of a population bottle technique six autosomal recessive mutants (ey^2, cl, vg, th, h, b) in Drosophila melanogaster were tested for heterozygote superiority (overdominance). The populations were maintained for at least twenty-five generations. With one possible exception none of the mutants maintained a significant excess of heterozygous individuals for the entire length of the experiment. It is, therefore, concluded that no evidence for overdominance was demonstrated in these experiments. An excess of heterozygotes was present, however, in the early counts. This latter fact is attributed to the phenomenon of multilocus heterosis. It is reasonable to assume that some inbreeding degeneration has occurred in a stock that has been maintained for hundreds of generations. In the event of such inbreeding degeneration in two different stocks, considerable heterosis is to be expected in the hybrid individuals. All first generation homozygotes for the mutant gene or its normal allele would be inbred like their parents. The offspring of these first generation homozygotes, like all F_1 offspring, might also be largely hybrids and hence more frequently heterozygous for the mutant than would otherwise be the case. Excess heterozygosis due to this cause might be expected to continue until the generation of flies is bred whose parents were only rarely derived from a single one of the two parent cultures. — In the thread experiment an excess of heterozygotes persisted for almost the entire experiment. Yet the frequency of the thread allele continually decreased and the degree of significance of the excess heterozygotes likewise decreased. It is postulated that this excess of heterozygotes is due to genes closely linked to thread and that recombination is breaking up this linkage as the experiment progressed.

HINTON, TAYLOR, University of California at Los Angeles. The genetic analysis of a nucleic acid requirement in Drosophila. — Standard crosses failed to reveal with certainty the location in the chromosome complement of the basis for the requirement since it behaved as a dominant effect in some crosses and as a recessive in others. Chromosomes

from a non-requiring strain were combined in all possible combinations with the chromosomes from the requiring strain. The results showed definitely that the basis for the requirement was on the same second chromosome with an inversion involving heterochromatin which produces a malformed eye. Certain genetic modifiers were found which affected both the eye phenotype and the requirement. Thus it appeared that both effects were position effects of the inversion. To test this point, a series of reversions and partial reversions of the eye phenotype was studied to determine the nucleic acid requirement. All 18 cases studied still had the requirement even though cytological studies showed that in every case, through subsequent rearrangement, the originally displaced heterochromatin had been moved to another position in the chromosome complement. In fact, the only factors held in common by these 18 strains and the original inversion was the requirement for nucleic acid and the displaced heterochromatin. It is, therefore, suggested that even though the original eye phenotype was caused by the proximity of heterochromatin to a particular region of the chromosome, the genetic basis of the nucleic acid requirement is a property of this heterochromatin itself and the effect remains regardless of the position of that heterochromatin so long as it remains altered.

HOROWITZ, N. H. and MARGUERITE FLING, California Institute of Technology, Pasadena, Calif. The autocatalytic production of tyrosinase in extracts of Drosophila melanogaster. — As a preliminary to an investigation of tyrosinase genetics in Drosophila we have begun a study of the enzyme in the wild type. Adult flies of the Canton-S stock are used. Fresh extracts are devoid of tyrosinase activity, but on standing at 0° they become active by a process which exhibits typical autocatalytic kinetics. This finding suggests a model of the trypsin-trypsinogen type, in which tyrosinase is formed by the tyrosinase-catalysed oxidation of one or more phenolic groups in a precursor. Tests of this hypothesis have shown it to be inadequate, since (a) activation occurs under conditions in which tyrosinase is inactive, and (b) the addition of tyrosinase (obtained by centrifugation of an activated extract) to a fresh extract does not show the expected effect on the rate of activation, whereas the supernatant, from which most of the tyrosinase has been removed, causes a marked increase. The results can be accounted for by the following model: Precursor + Activator = 2 Activator + Tyrosinase. According to this hyporhesis, tyrosinase is a by-product of an autocatalytic reaction in which it takes no part. — The reaction is very sensitive to pH, with the optimum at pH 6 when the extraction is made in water and at pH 6.8 when it is made in saline. This difference is due to the extraction by saline of a thermolabile factor which causes the pH-optimum of activation to shift toward higher values. — The Drosophila activation process appears to differ from that which has been described for grasshopper tyrosinase.

HOWE, H. BRANCH JR., University of Wisconsin, Madison, Wisc. Crossing-over in the first (sex) chromosome of Neurospora crassa. — Three regions: I, sex-ad-5 (71104), 4.6 units; II, ad-5-centromere, 2.5

units; III, centromere-vis (3717), 6.1 units are considered. Region IV, rib-1 (51602t)-centromere, 1.4 units, on chromosome 6, is used to detect nuclear transposition (e.g., slippage or spindle overlap). The cross is ad-5, vis, rib-1 A x wild type (73a-10a). — Of 940 asci dissected, enough spores germinated in 847 asci to allow complete tetrad analysis. The numbers of non cross-over and single cross-over asci observed agreed with calculated values. Of 19 double cross-overs found, 6, 9, and 4 involved 2, 3, and 4 strands, respectively. No triple cross-overs occurred. Although 5 of the 6 2-strand doubles involved regions II-III, directly across the centromere, four of these asci also showed apparent postreduction of rib-1 and therefore almost certainly resulted from nuclear transposition. No other double cross-over asci showed apparent postreduction of rib-1. The corrected doubles ratio, 2:9:4, differs significantly from Lindegren's (Genetics 27: 1-24) but not from the 1:2:1 chance expectation. The uncorrected doubles ratio does not differ significantly from Lindegren's, however, and this correction for nuclear transposition may account in part for his reported excess of 2-strand doubles across the centromere.

HSU, T. C., University of Texas, Medical Branch, Galveston, Texas. Abnormal mitosis in neoplastic cells and its implications on dynamic cytology. — Phase contrast, time lapse motion picture sequences of anomalous mitosis in HeLa (a tissue culture strain originally derived from a human cervical carcinoma) will be shown. There are many interesting features which cannot possibly be anticipated by examining fixed and stained preparations. For instance, a bipolar spindle may change into a tripolar arrangement during metaphase, telophasic chromosome groups may reunite into one nucleus, cytokinesis may delay for a long time after telophase, two widely separate cells may join together and form a single metaphase plate, etc. Rocking motion of nuclei before mitosis (pre-prophase) can be noted in a number of cases. These data indicate that tissue culture methods can be employed to reveal a number of hitherto unknown facts in cytology and that the dynamics of living cells needs further exploration.

HUESTIS, R. R. and RUTH S. WILLOUGHBY, University of Oregon, Eugene, Oregon. Neonatal jaundice in Peromyscus. — An inherited jaundice of the new born mouse has been discovered in our colony. Affected individuals are found to be yellow, or yellowish, a few hours after birth and the jaundice is followed by pallor and some debility. Some affected mice die or are destroyed by the mother, but recovery and later normal growth and fertility has been the rule. There is variability in the severity of the symptoms but penetrance is sufficient to make the identification of affected mice relatively easy. All of 8 mice jaundiced at birth, and examined at three months of age or later, proved to have splenomegaly. The spleen is characteristically black in color and roughly twice the size of the normal spleen in each diameter. Affected mice of breeding age have varying reticulocyte counts which average about 40 percent and do not overlap the counts made of normal mice of similar age. — The syndrome appears to be due to a single recessive gene substitution. Young mice not jaundiced after

birth but having one affected parent, do not have splenomegaly or a high reticulocyte count. Test crosses of similar mice in adequate number have given close to a 1:1 ratio of jaundiced and not jaundiced young.

HYDE, BEAL B., Botany Department, Indiana University, Bloomington, Indiana. Mitotic coiling of the differentiated chromosomes of P. ovata. — The structure of the telophase, interphase and prophase prochromosomes of this species has been studied in detail. Since the central segment of each of the eight diploid chromosomes never entirely loses its staining capacity, its coiling cycle can be followed through the interphase stage. The observations have been interpreted in terms of a conventional coiling scheme involving a single somatic spiral. The main difference between the coiling of the middle segments and the ends of the chromosomes appears to be a matter of timing. In the prochromosomes the old coil ravels more slowly while the new coil appears earlier than in the end segments. The new coil superposed on the old in various degrees of unwinding results in the great variety of shapes characteristic of prochromosomes. Partial uncoiling of chromosomes at several stages by ammonia vapor treatment indicates that chromomeres (in accordance with Rio and Crouse, P.N.A.S. 31: 1945) can be resolved into gyres of the coil. The constant structural differences along P. ovata chromosomes are believed due to constant differences in amplitude and frequency of the gyres of the coil.

JAMES, ALLEN P., Atomic Energy of Canada Ltd., Chalk River, Ont. Evidence of irradiation induced somatic crossing over in diploid yeast. — The gene pairs at three different loci, each concerned with galactose utilization (G_1g_1, G_2g_2 and G_3g_3) are being used to study a class of variants that occur with high frequency in diploid yeast following ultraviolet irradiation. The dominant phenotype is characterized by black (positive) colonies, the recessive by white (negative) colonies, on galactose-EMB indicator medium; and the absence of a dominant allele at any one of these loci results in the recessive phenotype. When positive cells that are heterozygous at one or more of these loci are irradiated and grown on indicator medium, variant colonies (either wholly negative or sectored) result. The variant frequencies are characteristic of the heterozygous loci, and at low doses of ultraviolet (200 ergs per mm^2) are 3.6, 1.7 and 1.0% in the genotypes G_3g_3, G_1g_1, and G_2g_2 (where the other loci are homozygous dominant). Three observations suggest that the variations are probably due to somatic crossing over: (1) in the sectored variant colonies from the Gg heterozygote there is a tendency for GG positive sectors to occur together with gg negative sectors, (2) variant colonies only rarely result on plating of irradiated positive haploid cells, and (3) the locus most frequently affected (G_3) is furthest removed from the centromere, as indicated by segregation studies of sex and galactose.

JANICK, JULES and E. C. STEVENSON, Purdue University, Lafayette, Ind. The effects of polyploidy on sex expression in spinach. — Crosses involving diploids and tetraploids were made to determine the effects of vari-

ous doses of X and Y genes on sex expression in spinach. Doubling of the chromosome number had no effect on the sex expression of staminate, pistillate, or monoecious plants. However from diploid x tetraploid and tetraploid x tetraploid crosses it was apparent that a single dose of Y causes the plant to be staminate even in combination with three doses of X. From the genetic ratios obtained, there is evidence that the disjunction of chromosomes containing the X and Y factors is completely at random in triploid and tetraploid plants. Triploid spinach was found to be highly fertile.

JANICK, JULES and E. C. STEVENSON, Purdue University, Lafayette, Ind. Genetics of the monoecious character in spinach. — The monoecious character in spinach appears to be controlled by a single "switch" gene. This gene, termed X^m, was found to be allelic to the gene or complex of genes, XY, conditioning the dioecious habit. X^m is incompletely dominant to X because plants of the genotype X^mX, although monoecious are more highly pistillate than plants of the genotype X^mX^m. The Y allele is completely dominant to X^m and X for plants of the genotype X^mY or XY are staminate. True-breeding monoecious lines (X^mX^m) may be inbred with high and low values of femaleness as measured by the proportion of pistillate to staminate flowers per plant indicating the presence of modifying factors.

KALTER, H., McGill University, Montreal, Quebec, Canada. Preliminary studies on the metabolic factors involved in the production of cleft palate in mice. — The administration of cortisone to pregnant mice of susceptible inbred strains at appropriate times during pregnancy induces a high frequency of cleft palate in the offspring. In continuing the investigation of this phenomenon, the writer has concentrated on clarifying the metabolic mechanisms involved by attempting to simulate cortisone's protein catabolic effect. — This was done by fasting pregnant females. Two days of fasting caused no abnormalities; 3 days a low incidence of cleft palate in the offspring of young animals and a much lower incidence in those of slightly older animals. To overcome the apparent refractoriness of the older animals to 3 days of fasting, a subteratogenic dose of cortisone was given on the first day of the fast. This, indeed, did have the desired effect—it increased the incidence and did so in older animals; but nor for long. As the females further aged, so even this treatment became less effective. — The hypothesis has been made that the protectiveness of age in this regard is due to increasing weight. Based on this, work now in progress is directed toward limiting weight increase during pregnancy, by restricting food intake prior to the fast, and toward preventing fat breakdown, thus enhancing protein breakdown, during the fast, by administering insulin at that time. If these treatments maintain or increase the frequency of starvation-induced cleft palate, the hypothesis is supported.

KENWORTHY, WALTER, Brown University, Providence, R. I. Effect of oxygen concentration on the survival rate of irradiated Habrobracon

eggs. — Survival ratios were determined for 3,998 Habrobracon eggs x-rayed during meiotic metaphase I in oxygen, air, or nitrogen. Survival of eggs irradiated in nitrogen ranged from 64.6% at 506 r to 10.6% at 2,200 r. Survival of eggs irradiated in air ranged from 36.3% at 506 r to no survivors at 2,200 r (1.9% survived at 1,518 r). Survival of eggs irradiated in oxygen ranged from 27.5% at 506 r to no survivors at doses of 1,518 r and above (2.6% survived at 1,100 r). — Survival ratios were determined for 1,622 Habrobracon eggs irradiated during meiotic prophase in oxygen or nitrogen. Survival of eggs irradiated in nitrogen ranged from 92.7% at 1,700 r to 20.0% at 24,000 r. Survival of eggs irradiated in oxygen ranged from 70.7% at 1,700 r to 1.6% at 24,000 r. — Dose-action survival curves for eggs irradiated in metaphase were exponential regardless of the gas in which irradiation took place. Dose-action curves for eggs irradiated in prophase were linear for the x-ray doses given. — Cytological studies of eggs irradiated with 1,000 r during meiotic metaphase I showed no chromosomal abnormalities other than terminal deletions. The percentage of such abnormalities was lower in eggs irradiated in nitrogen than in those irradiated in air or oxygen. Comparisons of chromosomal damage with survival data suggest that both dominant and recessive lethals decrease when irradiation takes place in the absence of oxygen.

KIMBALL, E., Clinton Experimental Farm, Clinton, Conn. Linkage in primary plumage patterns of the fowl. — Heritable units associated with primary plumage patterns determine gross phenotype, elaboration by melanocytes of specific eumelanin, and expression, modified expression, or nonexpression of secondary patterns. Experimental evidence indicates the heritable units are gene-clusters, comprising at least three chromosomal points, i.e., determinants of pigmentation, restriction, and down plumule length. Most probably, additional genes will be identified as components of the cluster. The situation is comparable to that encountered in pattern inheritance in insects, snails, fish, reptiles, and mammals (rodents); and a significant analogy exists with inheritance of blood groups (Sheppard, Amer. Nat. 87: 283-294, 1953). Species of Gallus, and subspecies of gallus, have a universal recessive (b-black) in common, linked with dominant pyle-zoned restriction and dominant long down. Wide diffusion of striped down phenotype and adult variants of R-restriction in the superfamily Phasianoidea, and striking analogies with larval, juvenile, and adult pattern successions in reptiles, e.g., Agama, suggest hoary antiquity for the Gallus type of gene-cluster. Polymorphic patterns in domestic fowl are demonstrably nonadaptive products arising incident to artificial selection. Although Gallus gallus mutates readily in respect to pattern under domestication, wild populations are peculiarly free of such novelties. If the gene-cluster of wild populations represents selection for increased linkage between genes affecting mutual selective values, and suppression of chiasma formation ensued by selection for inversions, it follows that nonadaptive mutants would be rapidly eliminated by natural selection. Conversely, selection pressure expressed as hominid whim would tend to preserve the same mutants in populations of domestic fowl, accounting for persistence of colum-

bian, extended black, birchen, quail, and other mutant primary patterns under artificial selection.

KIMBALL, R. F. and NENITA GAITHER, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Lack of an effect of a high dose of X rays on aging in Paramecium aurelia, variety 1. — It has been known for many years that Paramecium aurelia undergoes a progressive decline in division rate when it fails to undergo periodic nuclear reorganizations such as autogamy, A priori, this decline might be attributed to the accumulation and gradual segregation of deleterious mutations in the amitotically dividing macronucleus. A direct test of this hypothesis was made by exposing paramecia in the two-anlagen stage of postautogamous development to 80,000 r of X rays in order to induce a large number of mutations in the macronucleus. Thirty controls and thirty irradiated animals were used to start lines of descent which were continued as daily isolation lines with daily counts of the number of animals and staining of a sample from each line to check for autogamy. Each line was continued until it either died or underwent autogamy. After the first day, the division rate in the controls and irradiated groups became the same and remained the same for the duration of the experiment. After 44 days too few lines remained to furnish an adequate sample. The division rate declined linearly with time at the same rate in the two groups and reached about 70% of the starting rate in 44 days. Thus spontaneous macronuclear mutation is probably not the cause of the decline in division rate. Also a single large dose of X rays has no detectable effect on the aging process in vegetatively reproducing paramecia.

KING, R. C. and EUNICE M. Wood, Brookhaven National Laboratory, Upton, New York. Sex-linked lethal mutations induced by thermal neutrons in male and female Drosophila melanogaster.* — A study was made of the sex-linked recessive lethal mutation rate induced in the germinal tissue of Drosophila melanogaster males and females by thermal neutrons. The recessive lethal mutation rate/thermal neutron dose relation appears to be linear for sperm up to the highest dosage tested ($4.6 \times 10^{13} n_{th}/cm^2$). The relation is also linear for oöcytes and oögonia for doses up to 3.5×10^{13}-n_{th}/cm^2. The average mutation rate per unit dose for oöcytes is 75 per cent the male rate; for oögonia 38 per cent the male rate. The mutation rate in the most mature egg cells appears to be similar to the rate for sperm. To explain the lower frequency of mutations recovered from oögonia than from oöcytes it is assumed that either the mutation process occurs at a lower frequency in oogonia than in oocytes, or that a large fraction of the potential recessive lethal mutants are drawn off into inviable chromosome recombinations. On the basis of energy liberated per unit weight of gonadal tissue, thermal neutrons are found to be 2.5 times as effective as 90 kv X-rays in inducing sex-lined recessive lethal mutations in sperm and 1.8 times as effective in inducing mutation in oöcytes and oögonia. This greater efficiency is not related to the higher mean ionization density of the nitrogen capture protons which form the physical basis

of the action of this radiation. More likely the increased efficiency is due to a greater than average nitrogen content for the Drosophila gonad. (*Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

LEDERBERG, ESTHER M., University of Wisconsin, Madison, Wis. The inheritance of lysogenicity in interstrain crosses of Escherichia coli. — Of 50 diverse fertile strains, four proved to be sensitive to and lysogenized by the bacteriophage lambda carried by strain K-12. Crosses within these strains indicate an Lp locus determining lysogenicity and linked to Gal as in K-12. Each new strain lysogenized by lambda shows a more limited output of plaques when tested on K-12 than on the other sensitive indicators. Similarly, K-12 is more resistant than the other sensitives to free lambda originating from the other strains. By testing for sensitivity to both sources of lambda, and for lysogenicity on each indicator, four phenotypes are delineated: two lysogenic and two sensitive. These relationships are analogous to the host-induced modifications of lambda described by Bertani and Weigle (J. Bact. 65, 113). Whenever lysogenic x sensitive crosses involve K-12 as one parent, all four possible recombinant classes are found in the progeny, thus establishing a second locus, Mp, which modifies the expression of Lp. K-12 occurs as Mp^rLp^+ (lysogenic) or Mp^rLp^S (sensitive); the other four strains as Mp^SLp^+ (modified lysogenic) or Mp^SLp^S (modified sensitive). Linkage of Mp to the Lp-Gal loci was not demonstrated. Crosses reciprocal with respect to F (compatibility factor) differed in yield but not in the type of recombinants. The absence of sensitives from crosses of lysogenics segregating for Mp makes it likely that lambda prophage remains fixed to Lp, rather than Mp, in all lines.

LEDERBERG, J., University of Wisconsin, Madison, Wis. Phase variation in Salmonella. — The flagella carried by cells of a given serotype occur in two alternative phases (specific/group or 1 and 2) which are genetically conservative. The alternation may occur at a rate of 10^{-4} per generation (B. Stocker) or often much less, and superficially resembles point mutation. Genetic transduction analysis (Lederberg and Edwards, J. Immunol. 71, 232) has shown, however, that the alternative specificities are controlled by two distinct loci, H_1 and H_2, corresponding to the two homologous series of antigens, and accounting for the oscillation between just two states. The mechanism of genetic differentiation of the phases has not been settled: it might depend on the cytoplasm (as in Paramecium) or on the state of a third locus. However, the correlation found between the antigenic state of the donor cells and the transductive competence of phage lysates from them suggests a third alternative: that the differentiation is based on the states of the H_1 and H_2 loci themselves. — In addition, certain other antigenic variations, so-called "artificial phases" have been found to behave not as phasic oscillations but as point mutations of serological specspecificity, e.g. H_1^b to H_1^z33.

LEFEVRE, G., JR., and P. C. FARNSWORTH, University of Utah, Salt Lake City, Utah. Mutational isoallelism at the yellow and white loci in

Drosophila. -- Timofeeff-Ressovsky (1932) reported the existence of 2 different wild-type alleles of white (w^{+A} and w^{+R}), distinguishable only by the fact that after irradiation w^{+A} mutated to w more than twice as frequently as did w^{+R}. We have verified this mutational isoallelism at the w^+ locus, and also have detected the same phenomenon at the yellow (y^+) locus. -- Males containing y^+ or w^+ or both from 6 different stocks were exposed to 5000r doses of X-rays, and from 25,000 to 65,000 F_1 females in each series were examined for y and w mutations. Male-viable, male-lethal, and sterile mutants were recorded. The sterile mutants were apportioned among the other 2 classes, and then the expectancy of male-viable y and w mutants per 10^5 gametes tested was calculated. Such mutants fell into 2 distinct, non-overlapping mutability categories. Published mutation rates (including Timofeeff's) from similar experiments showed surprisingly good agreement. Adjusted to 5000r, male-viable y mutants occurred at the following 2 rates: 7.8 and 17.8 per 10^5; similar w mutants at 34.4 and 79 per 10^5. These values suggest the occurrence of a mutation "unit" of 8 or 9 per 10^5 ($1.6 - 1.8 \times 10^{-8}$ per gamete per r). Less extensive data at the vermilion and forked loci show only one mutation rate, similar to the low white rate. Perhaps in different stocks pseudoallelic duplication has occurred to different degrees; i.e., y^+ or $y^+ y^+$, $w^+ w^+$ $w^+ w^+$, etc.

LEVITAN, M., Virginia Polytechnic Institute, Blacksburg, Va. Additional evidence of position effects in natural populations. -- Local populations of D. robusta contain the four possible combinations of two gene arrangements of the left arm of the X-chromosome, XL and XL-1, and two right-arm arrangements, XR and XR-2. The frequencies of the combinations are generally not equal to the frequencies expected on the assumption that left- and right-arm arrangements are independent. In the total data chromosomes XL XR and XL-1 XR-2 consistently and significantly exceed expectation, XL and XR-2 and XL-1 XR are deficient. However, the deviations from random association are not found in every season; nor are they uniformly significant in the two sexes or in both egg and adult samples of the same sex. It follows that selection is responsible for these results. The phenotypes with different adaptive value could stem from interaction of loci on the left- and right-arm arrangements, or they could stem from differences in the positional relationships of genes in the arrangements. The latter is the more likely hypothesis, as indicated by the greater number of adult females with linkage type XL XR/XL-1 XR-2 than with XL-1 XR/XL XR-2. If the position of the genes were not a factor, these types should be equal. Together with evidence of position effects of second chromosome inversions previously noted (in press), these data suggest that the role of position effects of inversions in natural populations has heretofore been underestimated.

LEWIS, H. W., University of California, Berkeley, California. Studies on a melanoma-producing lethal in Drosophila. -- Experiments have been performed to study the action of a sex-linked, recessive gene in Drosophila melanogaster which in the hemizygous condition results in tumor formation

and death during the late larval period. Evidence from gene dosage experiments indicates that each dose of the lethal gene has an additive effect on tumor development. The viability of tumorous larvae is reduced by both genetic and environmental manipulations. Quantitative measurements of the effects of temperature and crowded conditions have been made and evidence of genetic modifiers has been found in four out of 23 wild type stocks tested. Heterozygotes for the lethal have a 3.7% increase in rate of development and are endowed with increased viability. The superiority of heterozygotes for the lethal over the wild type in a highly competitive environment has been measured in a population bottle experiment. Using paper chromatography, free amino acids of the body fluids of hemizygous, heterozygous, and duplication larvae were compared. Tumorous individuals show an absence of cystine and have higher levels of alanine, arginine, glycine, methionine, serine, and tyrosine than their controls.

LINDEGREN, C. C. and E. E. Shult, Southern Illinois University, Carbondale, Illinois. A general theory of crossing-over. — A theory of crossing-over has been formulated without making the demonstrably incorrect assumption that low frequency of recombination means low frequency of crossing-over. An algebra of tetrad analysis was based on three elements —the respective frequencies of (1) parental type, (2) recombinant type, and (3) tetratype tetrads. The possible effects of sister-strand exchange and chromatid interference upon the frequencies of different types of tetrads were analyzed by considering the effect of a single cross-over inserted to the right of a postulated cross-over pattern and calculating the limits of continued insertion of such cross-overs. It has been possible to show that sister-strand crossing-over either does not occur or occurs at a very low frequency and that chromatid and chromosomal interference generally have very low values. The relatively high frequency of recombinant tetrads indicates that crossing-over at the two-strand stage is not uncommon. The exceptionally high frequency of recombinant tetrads in some dihybrid analyses could only be explained on the assumption that crossing-over at the 2-strand stage occurred at every meiosis in one short region of the chromosome. The high frequency of recombinant tetrads in some dihybrid analyses indicates that the order of members of a linkage group is determined by minimizing the frequency of random recombination between the loci involved. If crossing-over occurs at the two-strand stage, previous theories of crossing-over are inadequate. A new theory of crossing-over is proposed on the hypothesis that crossing-over occurs at both the two- and four-strand stage.

MARKERT, C. L. and GLENN FISCHER, University of Michigan, Ann Arbor, Mich. Melanogenesis in cells of diverse genotype cultured in vitro. — The type of melanin (black, brown, or yellow) synthesized by a melanoblast is determined by its genotype and embryological history. The role of cell genotype in determining the substrate used for melanin synthesis was studied by culturing melanoblasts in vitro in the presence of C14 labeled tyrosine, tryptophane, dopa, and unidentified tyrosine oxidation products.

Melanoblast-containing tissue from New Hampshire Red and Barred Rock embryos and from inbred strains of black, brown, and yellow mice embryos were cultured in vitro until numerous melanocytes developed. The cultures were then prepared by conventional techniques for histological examination, coated with plastic, and radioautographed. Although the tissue cultures were saturated with labeled tyrosine (about 0.1 μC per hanging drop culture) during the period of melanogenesis no selective accumulation of tyrosine in melanocytes occurred in any of more than 100 cultures radioautographed. Similar concentrations of labeled dopa also failed to yield selective radioautographs in more than 30 preparations tested. Tryptophane completely inhibited melanogenesis. However, distinct radioautographs were obtained of melanocytes that developed in the presence of oxidation products of tyrosine. These oxidation products of tyrosine were obtained by incubating tyrosine, tyrosinase, and ascorbic acid until dopachrome was formed at which time the reaction was stopped by denaturing the enzyme. Thus, under the conditions of tissue culture, melanogenesis does not involve the selective uptake of exogenous tyrosine, tryptophane, or dopa, but does involve the selective concentration of some product of tyrosine oxidation. The role of cell genotype in substrate utilization will be discussed.

MARTIN, A., JR.[1], and R. M. WOTTON, University of Pittsburgh, Pittsburgh, Pa. The Golgi bodies as indicators of a common genotype. — Evidence is being accumulated to support the hypothesis that many genes (some 70-80 per cent of the genotype) are identical in structure and function in all living cells. Our observations on the Golgi substance indicate that it consists of a complex enzyme system containing a skeleton protein, and that this Golgi substance is active in the metabolism of fat. Observations on cells from oil-fed mamals, birds, reptiles, amphibians, and fish indicate that lipids are directly absorbed through cell boundaries, and that the conversion of such lipids into living protoplasm within the cell takes place through the instrumentality of the Golgi substance. The close physical association between the Golgi vesicles and the absorbed fat together with associated changes in the chemical composition of the absorbed fat while so associated, indicates that the Golgi substance is essential for the transmutation or conversion of foreign fat into species-specific fat. To be sure, the concept of species specific fat is not new, but as our cytological investigations demonstrate the Golgi vesicles are operative in building cytoplasm. While it is not easy to follow the course of water soluble amino acids and carbohydrates in metabolism, these substances, like fats, may also be processed by the Golgi substance before being ultimately incorporated into the living state. The morphology of the Golgi apparatus during fat metabolism is similar in the representative vertebrates studied. Such close similarity in all species examined would seem to indicate a common role and therefore a common genotype for the Golgi substance in fat metabolism throughout the Vertebrata. ([1]Veterans Administration Hospital, Leech Farm Road, Pittsburgh 6, Pa.)

MEYER, HELEN U., Indiana University, Bloomington, Ind. Crossingover in the germ line of Drosophila melanogaster males following irradiation of the embryonic pole cells with ultraviolet.* — Crossingover in males of Drosophila is extremely rare; its frequency, influenced by certain physiological conditions, has been shown to be considerably increased by several mutagenic agents, particularly by certain ionizing radiation. — Several such cases had likewise been found in our earlier work applying ultraviolet to the embryonic pole cells, many of which enter the germ line. The distribution of recombinants indicated their origin long before meiosis, possibly caused by the treatment. — New experiments were performed using doses between 200 and 400 ergs/mm^2 of ultraviolet (2537 Å). Our most extensive data are for exchanges between the right arms of chromosomes 2, comparing some 34,800 offspring from 256 treated males and 98,600 from 624 controls. An 8.6-fold increase in the treated lot was found, with a frequency of .129% against .015% in controls. — Multiplicity of identical crossover offspring was similar to that of the lethals often induced in these same males, but crossovers were found more frequently in later broods. — 2 crossover events among controls yielded 11 and 4 crossovers out of 181 and 118 offspring, respectively, while 10 cases in the treated group had 45 crossovers in 1840 offspring. — When calculating the frequency of crossover events on the basis of number of treated primordia, one has to allow for some 2.9 times as many pole cells surviving in control embryos as in the treated lots, with these particular doses. Taking this into account, one arrives at a 30-fold increase after ultraviolet treatment. (*This work was supported by a grant received for work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

MEYER, JAMES R.*, Delta Branch Experiment Station, Stoneville, Miss. Genes from cotton species. — Gossypium hirsutum, or upland cotton, is an allotetraploid species ($n = 26$) with sets of A chromosomes from linted Asiatic diploid cotton ($n = 13$) and sets of D chromosomes from lintless American cotton ($n = 13$). Pentaploid hybrids have been produced by crossing upland cotton with diploid A or D species, doubling the chromosome number of the triploid hybrid, and backcrossing the resulting hexaploid to upland. The use of pentaploid hybrids enables one to move or transfer definite genes from definite diploid species to upland cotton. A "doubled haploid" of upland, which is completely homozygous, has been used in the synthesis of these pentaploids and their derivatives. Fourteen different plant, leaf, and flower characteristics due to dominant or partially dominant genes are being transferred to this doubled haploid by backcrossing. The resulting isogenic strains can be used for interspecific genome analysis and other studies. (*Cytogeneticist, Delta Branch of the Mississippi Agricultural Experiment Station and Cotton Section, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, cooperating. — This abstract was prepared as part of a study which has been supported in part by funds appropriated under authorization of the Research and Marketing Act.)

MICKEY, GEORGE H., and ARMON F. YANDERS, Northwestern University, Evanston, Illinois. The production of dominant Minutes in Drosophila sperm irradiated with X rays, gamma rays and fast neutrons. — Mature sperm of Drosophila melanogaster (Oregon-R) were given doses ranging from 1500 to 9000 r of either Co-60 gamma rays of 250 kvp X-rays or from 250 rep to 2000 rep of fast neutrons. Treated males were mated to wild type virgins and transferred to fresh cultures each day through four cultures. The dominant Minutes were detected in the F_1 flies. Rates of Minutes induced by these high energy X rays did not differ statistically from those induced by gamma rays. The fast neutrons, however, were much more efficient per unit of dose in producing Minutes; their R. B. E. was about 4.5. Measured in terms of Minutes induced, the effects of these agents appear to be directly proportional to dose and also related to specific ionization density of the path. (Work supported by a research grant from the United States Atomic Energy Commission, Contract No. AT(11-1) -89, Project No. 7)

MILLER, D. D., University of Nebraska, Lincoln, Nebr. Intraspecific variation in spermatheca morphology in Drosophila affinis Sturtevant. — Spermatheca morphology was observed in strains of D. affinis from Florida, Illinois, Massachusetts, Nebraska, Tennessee, and Texas. External shape was found to vary from nearly spherical to relatively flat (strain mean averages of ratio of spermatheca width to height ranging from 1.22 to 1.61). There was also found to be variation in degree of penetration (telescoping) of the tube into the spermatheca (strain mean averages of ratio of spermatheca height to length of telescoped part of tube ranging from 1.34 to 2.84). In addition, the spermathecae sometimes had a pronounced terminal indentation, frequently only a slight indentation or none at all (strain frequencies of presence of terminal indentation ranging from 96% to 0%). — Reciprocal crosses were made between members of the Florida strain (characterized by relatively flat spermathecae, much penetration of the tube, and very pronounced terminal indentations) and members of the Texas strain (with nearly spherical spermathecae, little tube penetration, and no terminal indentation). The F_1 and F_2 generations had intermediate means of the spermatheca dimension ratios and the frequencies of terminal indentations, with the F_2's somewhat more variable than the F_1's. Backcrosses to the parent strains (all possible combinations) caused shifts in these values in the directions of the respective strains. No influence of direction of the original cross was apparent in these generations. The results are consistent with the interpretation that spermatheca morphology is governed by numerous nuclear genes, with little or no influence of extranuclear factors.

MILLER, WILMER J., University of Wisconsin, Madison, Wis. Segregation of species-specific antigens and the "hybrid substance" in backcross hybrids following a generic cross in Columbidae. — In contrast with the usual sterility of the hybrids from matings of the domestic pigeon, Columbia livia, with ring dove, Streptopelia risoria, eight offspring from a mating of an F_1 male to a ring dove have been obtained by a private breeder

who kindly made their bloods available to the author. Agglutination tests were made with reagents detecting cellular species-specific antigens (A′, B′, C′, and E′) of livia in contrast to both C. guinea and S. risoria, and with reagents detecting the "hybrid substance" which is present in all F_1 hybrids, but not in the parental species. — A segregation of the "hybrid substance" and of each of the species-specific antigens of livia was observed among these eight backcross birds. The "hybrid substance" was demonstrable only in backcross birds which also possessed the C′ substance of livia, suggesting an association with C′. — The species-specific antigen C of guinea is antithetical to C′ of livia; heterozygotes (CC′) have a "hybrid substance." Of several serological specificities demonstrated for the "hybrid substance" of F_1-livia/risoria, one was associated with C of guinea, and another with heterozygotes (CC′). The assumption is that the "hybrid substance" of F_1-livia/risoria birds results from some kind of interaction of the genes or gene-products responsible for C′ of livia and a C-like antigen of risoria.

MORGAN, WALTER C., University of Tennessee, Knoxville, Tenn. — Eventration and exencephaly in mouse embryos. — Mice heterozygous for for the dominant tail mutation Crooked (a semi-lethal) were mated to mice heterozygous for the dominant tail mutation Tail-short (a lethal). Relatively small litter-size of the F_1 suggested a prenatal lethality. Dissection of females during late gestation provided a high incidence of monsters. -- The abnormal mice were smaller than their normal sibs and many were tailless. Conspicuous cranial overgrowths (exencephaly) and eversion of viscera (eventration) were observed in a higher proportion of the embryos than had ever been reported from dissections involving either of these mutations alone. All of the embryos with exencephaly and/or eventration either had very short tails or were tailless. This phenotype is representative of the Ts/+ genotype and not of Cd/+. Although exencephaly has been reported in Cd/Cd individuals, it has not been observed in the heterozygotes. — The high incidence of grossly abnormal embryos suggests an interaction of Cd and Ts which abruptly disorganizes normal embryonic development.

MORSE, M. L. (Introduced by M. R. Irwin.), University of Wisconsin, Madison, Wis. Transduction of certain loci in Escherichia coli K-12. — Lysogenicity for the phage lambda is determined by a nuclear gene closely linked to a cluster of loci affecting galactose fermentation (Lederberg and Lederberg, Genetics 38, 51). A small fraction of the cells in galactose-negative cultures can be transformed to fermenters by lambda lysates from positive, or from non-homologous negative, cells. The interactions between cells and lysates are concordant with allelism tests by crossing. With excess assay cells the number of transformations is proportional to the amount of lysate added, with an efficiency of about one transduction per million plaque forming particles. Most transformed clones are unstable for galactose fermentation and continue to segregate galactose negative cells after many single colony isolations. When Gal_1^- cells are trans-

formed with wild type lysates the negative segregants from the "heterozygous" positives are Gal_1^-. When Gal_1^- cells are transformed with a lysate of Gal_2^- cells, the negative segregants are usually Gal_1^-, occasionally Gal_2^-, and rarely $Gal_1^-Gal_2^-$. Similar results have been observed with various combinations of Gal_1, Gal_2, and Gal_4. Exceptional lysates transduce with an efficiency greater than 10^{-1}. These lysates are capable of (1) transforming a large fraction of a cell population, (2) transducing Gal^- as well as Gal^+ alleles, and (3) showing that adsorption of lambda to a cell is necessary, but not sufficient for transformation. The phage here, as in Salmonella (Zinder and Lederberg, J. Bact. 64, 679), acts as a passive vector of genetic material. Other loci tested, not linked to Gal, are not transduced by lambda.

MULLER, H. J., Indiana University, Bloomington, Ind. Characteristics of the far stronger but "spottier" mutagenicity of fast neutrons as compared with X-rays in Drosophila spermatozoa. — Experiments of 1953, done with cooperation of Herskowitz, Abrahamson, Oster and others, taken in conjunction with earlier work with the Valencias and others (see these Records, 1951), establish frequency of translocations connecting second and third chromosomes at approximately $68 \cdot 10^{-6}$/rep, practically independently of dose, for neutrons from either Oak Ridge cyclotron or pile, applied to mature spermatozoa in young males. This effectiveness is 2.4 times that of 4000r X-rays, varying with X-ray (dose)$^{-1/2}$. — NXE (neutron: X-ray effectiveness) in inducing male exceptions, lacking either paternal sex-chromosome or its marked portion, was 3-5. However, subtraction of partial losses, estimated by tests, indicated that NXE for complete losses, presumably representing isochromatid bridges derived from individual breaks, was about 7. Note agreement between these results and those on dominant lethals (Russell et al, 1953; Baker and von Halle, 1954), all obtained independently. Causes of lower NXE for eucentric rearrangements than for individual breaks are: (1) neutron rearrangements contain more complications; (2) they are therefore oftener aneucentric; (3) neutron-induced fragments probably have slower joining because multiple neighboring breaks ("shattering") must impede unions between major pieces, resulting in isochromatids. — NXE for producing separately registered sex-linked recessive lethals was 2.4. However, for all visible changes of expression of specific loci NXE was about 4, as it was for proved "point-mutations" of these loci (this holds also for female germ cells). This difference from lethals is caused by multiple neighboring effects with neutrons, which hide some third of point-mutational lethals, yet cause neutron-induced rearrangements to affect more loci (hence, to give more "visibles") than X-ray-induced rearrangements. (Work supported by grants from American Cancer Society, on recommendation of Committee on Growth of National Research Council, and from U. S. Atomic Energy Commission (Contr. AT(11-1)-195).)

PALM, JOY and M. R. IRWIN, University of Wisconsin, Madison, Wis. A "hybrid substance" associated with a species-specific antigen in back-

cross hybrids. — A cellular antigen, d-4, peculiar to Pearlneck (Streptopelia chinensis) in contrast to Ring dove (S. risoria) has been obtained as a genetic unit in backcrosses to Ring dove of hybrids and selected backcross hybrids from matings of Pearlneck and Ring dove. Similarly, a d-4 like antigen, s-4, of Senegal (S. senegalensis) has been isolated following matings of Senegal and Ring dove. — Antisera produced against erythrocytes of backcross birds possessing only the d-4 antigen become reagents specifically reactive against d-4 after absorption with cells of Ring dove. These reagents agglutinate the cells of Pearlneck, Senegal and their backcross hybrids possessing d-4 or s-4. After absorption with pooled cells of Ring dove and Pearlneck, certain anti-d-4 sera reacted with cells of all backcross hybrids possessing d-4, indicating the presence of an antigenic specificity ("hybrid substance") on these hybrid cells not expressed in either parental species. This reagent also agglutinated the cells of Senegal and of its backcross hybrids containing s-4. — The new antigenic specificity in backcross birds possessing d-4 may result from (a) an interaction of the gene or genes producing the d-4 of Pearlneck (or of linked genes) and genes of Ring dove, or (b) some kind of antigenic interaction. The combination of the d-4 "hybrid substance" and a d-4-related antigen, s-4, on the cells of Senegal, and the segregation of these two specificities as a genetic unit in backcrosses of Senegal hybrids suggest that the "hybrid substance" associated with d-4 is under genetic control and is, therefore, probably the result of gene interaction in backcross birds with d-4.

PARKER, D. R., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Role of the Y chromosome in induced detachment of attached-X chromosomes in Drosophila melanogaster. — Radiation-induced detachment of attached-X chromosomes is a "two-hit" process (Parker, 1953; Herskowitz and Muller, 1953). If no Y chromosome is present, the exchanges are mainly X-4 translocations. The presence of a Y chromosome ($sc^8.Y$) increases the detachment frequency to about 1.7 times the frequency obtained without a Y. About 80% of the separations involve an exchange with the Y as determined by linkage of y^+, and by male fertility tests. The remaining 20% are mainly X-4 translocations. — Substitution of $sc.Y^L$ (a two-armed Y chromosome lacking Y^S fertility factors) gives no significant change in detachment frequency from that obtained using $sc^8.Y$. However, with $sc^{VI}.Y^S$ the rate is nearly doubled. With the ring chromosome Y^{LC}, the detachment frequency does not differ significantly from that obtained with no Y present, but segregation and/or type of exchange may be different. — It is concluded that there is close association between X and 4 in oocytes and that preferential pairing is such that the Y tends to "protect" chromosome 4, and Y^L tends to "protect" Y^S from exchanges with the X chromosome.

PAULEY, S. S., Maria Moors Cabot Foundation for Botanical Research, Harvard University, Petersham, Mass. Variation in time of break of dormancy among altitudinal ecotypes of Populus trichocarpa. — Altitudinal ecotypes of P. trichocarpa grown under uniform temperature and day-

length conditions during the spring of 1953, clearly indicated that low elevation (long growing season) types were significantly more precocious than high elevation (short growing season) types. These results were highly instructive, since we had anticipated that high elevation ecotypes would demonstrate a precocity similar to that which characterizes the behavior of their high latitude cousins.

PAULEY, S. S., Maria Moors Cabot Foundation for Botanical Research, Harvard University, Petersham, Mass. Influence of light on break of dormancy in various tree species. — In connection with studies of the influence of light on the break of dormancy in Populus ecotypes, ramets of various clones representing other species in other forest tree genera were also grown under uniform temperature in continuous light, natural day, and continuous darkness for a period of ca. 3 months in the late winter and early spring of 1953. Results of these studies reported previously for Populus (Jour. Arn. Arb. 35: 167-188, 1954) indicated that light or its periodicity apparently has little, if any, influence on break of dormancy in Populus. — Except that ramets exposed to continuous darkness were inhibited in varying degree (0-16 days), representatives of the following species broke dormancy in all compartments: yellow, paper, black, and gray birch; pin and black cherry; striped, red, and sugar maple; white ash. — Of special interest is the fact that members of the Fagaceae represented in the study (red, white, and black oak; American chestnut; and American beech) failed to break dormancy in continuous darkness. No significant difference in time of dormancy break was noted between ramets of these clones, or those noted above, in the continuous light and natural day compartments.

PITTENGER, THAD H. and K. C. Atwood, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. The relation of growth rate to nuclear ratio in Neurospora heterocaryons. — In heterocaryons involving biochemical mutants in Neurospora crassa it has been assumed that there is a continual selection for nuclear ratios which will give maximum rates of growth. It is possible to test this assumption by comparing growth rate with changes in nuclear ratios in the same heterocaryon with different nuclear ratios. Such heterocaryons can be produced experimentally by mixing conidial suspensions of two nuclear types in various proportions. Nuclear ratios as great as 30:1 have been produced by this method. Conidial mixtures of the desired proportions are placed in growth tubes and samples of the conidia removed at intervals. These conidia are plated on minimal and supplemented media to estimate the nuclear ratios. Strains tested in various heterocaryotic combinations included Pan, al-1(5531,4637), Nic, al-2(4540,15300), Lys(4545), Arg(29997) and Pan, Paba, al-1(5531, 1633, 4637). — The relation between the nuclear ratio and the growth rate was found to be variable depending on the heterocaryon involved and the initial nuclear ratio. In most cases the nuclear ratio remained constant at the initial values throughout the length of the growth tube and the growth rate was unchanged over a wide range of nuclear ratios. On the other hand, in some heterocaryons, regardless of the initial ratio, there was selection

for one of the nuclear types without apparent change in growth rate. In some cases the growth rates were equal but submaximal at several different nuclear ratios. In still another case the nuclear ratio of two strains mixed in several different ratios stayed at the initial value through 10 days of growth without any selection even though the growth rate at the most extreme ratio was less than half that of the same heterocaryon at other ratios. It is apparent that in many heterocaryons there is not a definite selection toward nuclear ratios which give maximum growth rates.

RAGLAND, J. B., Genetics Laboratory, Department of Zoology, University of Texas, Austin 12. A strain of Neurospora inhibited by histidine. — Strain T-66, obtained by ultraviolet irradiation, is strongly inhibited by L-histidine in the medium, although it approximates the 72 hour growth of wild type in minimal medium. Wild type shows a slight inhibition by histidine in the early stages of growth but is able to overcome this inhibition completely in 72 hours. Inhibition of T-66 is reversed by all amino acids tested except aspartic acid, lysine, proline, and hydroxyproline. No other biochemicals tested other than amino acids relieved the inhibition. In the absence of an inorganic nitrogen source T-66 is able to utilize all amino acids tested as nitrogen sources as well as wild type. If histidine is present with amino acids as the sole source of nitrogen only alpha-aminobutyric acid, phenylalanine, methionine, threonine, serine, leucine, isoleucine, and valine appreciably relieve the inhibition by histidine. Six day growth experiments with T-66 shows that this strain is able to overcome the histidine inhibition in an adaptive fashion and reach maximum weight in six days. Preliminary studies of glutamic-aspartic and glutamic-leucine transaminases in dialyzed extracts of both wild type and T-66 indicate that histidine interferes with transamination in Neurospora. These systems are now being further investigated and will be discussed. Although the exact nature of the mutation involved in T-66 is not known, it appears to have altered the metabolism of the organism in such a fashion that the presence of histidine upsets the nitrogen metabolism. (These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Texas, NR 859(00).).

RATTY, FRANK J., University of California, Berkeley. Some x-ray induced effects on embryonic viability in white leghorn chickens. — One effect of 1000 r. units of X-rays on the sperm of three consecutive generations of white leghorn chickens was a marked decrease in the viability of the zygotes following fertilization. This was interpreted on the basis of a genetic rather than a physiological action of the irradiation. When the proportion of viable 18 day embryos was determined, it was found that there was a depression of the viability in each generation from 71.0% in the controls to 28.5% in the first generation following irradiation to 21.9% in the second and 19.8% in the third. The viable 18 day old zygotes were not the result of fertilization by unaffected sperm. This was shown by the fact that when irradiation was omitted in the following generation, there was a residual loss of viability among their progeny to 61.3% in the first

and 52.5% in the second generation. This continued depression of the viability in subsequent generations indicated that inherited detrimental factors already present were augmented by additional treatment. The differential loss of viability observed between the zygotes produced by irradiated sperm and zygotes that were the result of non-irradiated gametes of the preceeding irradiated generation is best explained by the presence of dominant lethals in the former. However, the analysis of dose level experiments indicated that embryonic survival may be associated with recessive lethal effects as well as gross chromosomal changes.

RAUT, CAROLINE, Detroit Institute of Cancer Research, and Wayne University College of Medicine, Detroit, Michigan. The effect of ultraviolet light of various wavelengths on the production of cytochrome-deficient yeast. — Normal and cytochrome-deficient haploid strains of yeast were irradiated at various wavelengths of ultraviolet light. Both strains were killed at comparable rates with maximum rate of kill at 2600 Å. The action spectrum corresponds to the absorption spectrum of nucleic acid. A large proportion of the survivors of the irradiated normal cells produce cytochrome-deficient "petite" colonies. This cytochrome deficiency is cytoplasmically rather than genically determined. The production of petite colonies also exhibits a maximum at 2600 Å. It is suggested that absorption of ultraviolet by nucleic acid is involved in the destruction or inactivation of the cytoplasmic particles necessary for the production of the complete cytochrome system and possibly that the particles themselves contain nucleic acid.

RAY, DAVID T., FRISSELL HUNTER, and G. J. STEPHENS, Howard University, Washington, D. C. Sex difference and oxygen consumption in the wasp Mormoniella. — Rates of oxygen consumption in M. vitripennis were measured by the direct method of Warburg in microliters per gram per hour at various stages of development. Rate was highest in the eggs, 565.71, lowest in diapause larvae, 48.56. For males (from unmated females) and (85%) females (from mated females) respectively, the young larvae showed 533.33 and 559.06. These figures decreased to 334.71 and 287.67 on the sixth day just before pupation. For males and females respectively, rates were 320.14 and 217.16 at pupation, 133.56 and 130.60 after 60 hours, 218.39 and 182.27 just before eclosion, showing the U-shaped curve characteristic of most insects. Adult males and females gave 211.92 and 204.96 just after eclosion, 354.76 and 310.09 on the fourth day and 120.70 and 163.62 on the sixth day after eclosion. Graphically represented the rates show a rapid drop for the first two days to 408.25 in both sexes, followed by a gradual leveling off in which the males consistantly show a greater oxygen consumption, with the exception of one point during late pupation. This is probably the point of greatest gonad differentiation. Although males are smaller than females and much more active, both are sexually mature at eclosion. The data indicate sex differences in metabolic rate.

ROBINSON, H. F., R. E. COMSTOCK and P. H. HARVEY, North Carolina State College, Raleigh, North Carolina. Heterosis in crosses between open-pollinated varieties of corn. — With certain postulated conditions the dominant favorable gene hypothesis would not allow for gains of more than 5 per cent for hybrids between inbred lines above the random breeding populations from which the lines were derived (Crow, Genetics 33: 477, 1948 and Heterosis, Chap. 18, 1952). The demonstration of appreciable increases in heterosis in yield (where yield is essentially equated to selective value by Fisher (Theory of Inbreeding, 1949) and Crow (op. cit.)) in crosses of open-pollinated corn varieties is evidence against the validity of all of the assumptions made by Crow, applying to these random breeding parent populations. The presence of some overdominant loci has been suggested as a possible explanation of part of the heterosis occurring in hybrids of inbred lines. The overdominance hypothesis is not compatible with large amounts of heterosis in variety crosses if the genes in the parent populations are at equilibrium between mutation and selection. — The results to be presented from tests involving six open-pollinated varieties of corn and the fifteen possible crosses between these varieties are of interest with regard to the nature of gene action determining yield inheritance. Variety crosses yielded an average of approximately 20 per cent above the mean yield of parent varieties in two years at three locations. Certain combinations exceeded the mid-parent by more than 30 per cent. The use of reciprocal recurrent selection, with two open-pollinated varieties as foundation material, is suggested as a practical breeding procedure for improving both yield and agronomic characters of a variety cross.

RUNNER, MEREDITH N., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. Inherited hypofunction of the female pituitary in the sterile-obese syndrome in the mouse. — Sterile, obese females, homozygous for the recessive gene ob, have conceived only after artificially induced ovulations and matings. The earliest detectable symptoms of the hypogonadel portion of the syndrome have been investigated by comparing genetically obese mice with their nonobese sibs as controls. Tests for genetic competence of the uterus showed that (a) castration did not significantly change uterine weights in obese animals and (b) administration of estrogen to castrate and intact obese animals gave uterine responses comparable to those obtained in controls. Differences between uterine weights of obese and their normal sibs was apparently due to differences in levels of circulating estrogen. Genetic competence of the ovary to produce estrogen and/or to respond to gonadotropin was tested by administration of pregnant mare serum. The ob ovary proved capable of responding unusually well to gonadotropin and elaborated sufficient estrogen to render uterine weights in obese animals comparable to those in controls. — Hypofunction of the pituitary gland has been demonstrated, therefore, by (a) uteri of intact obese animals remaining castrate in size and (b) uteri and ovaries of obese animals being capable of adequate responses to administered hormones. The hypogonadal portion of the syndrome had its onset concomitant with phenotypic obesity, between 25 and 35 days of age. Vaginas of the

obese mice opened at the appropriate age but ovulation never followed and the animals remained prepuberal indefinitely. The cause of obesity in the adult mouse has been attributed to a primary block in acetate oxidation (Guggenheim and Mayer, 1952) and it is intriguing to speculate how this "hereditary biochemical lesion" might correlate with failure of the pituitary to elaborate gonadotropin.

RUSSELL, LIANE BRAUCH and R. JENELLE SPEAR, Oak Ridge National Laboratory, Oak Ridge, Tennessee. X-ray-induced dominant lethals in mouse oocytes and their relation to irradiation-to-ovulation interval. — Female (101 x C3H) F_1 mice were mated at various intervals after exposure to 400 r of X rays. Embryos were examined at the following postcopulation ages: (1) within 24 hours, alive under phase contrast; (2) at days 1, $1\frac{1}{2}$, 2, $2\frac{1}{2}$, in sections stained by modified Feulgen; (3) at 2 weeks, at which time corpora lutea were also counted. The number (per female) of living 2-week embryos is very low for matings made within 18 hours after irradiation, approximately normal for the next four days, then, after a sharp drop, decreases gradually to zero for matings made more than 32 days postirradiation. For the first 3 weeks postirradiation this decrease in living young is not due to decreased number of ovulations. From days 2 to approximately 16 postirradiation, ovulations are actually significantly increased (to about 150% normal at peak), and there is a proportionate increase in the number of ovulated eggs fertilized normally. Therefore, on days 2-16, irradiated females yield a higher absolute number of fertilized eggs and the normal and decreased littersizes represent death of embryos. The finding of nuclear fragments in blastomeres indicates that some if not all death is due to the induction in the oocyte of chromosomal changes with dominant lethal effects. The results cited indicate that the incidence of such effects is about three times as high when eggs are irradiated just prior to ovulation (presumably while in meiotic division I), as when they are irradiated during the earlier parts of that estrus.

SAND, SEAWARD A., HAROLD H. SMITH, and ARNOLD H. SPARROW, Cornell University, Ithaca, N. Y., and Brookhaven National Laboratory, Upton, N. Y. — Stimulation by chronic gamma irradiation of the spontaneous rates of heritable somatic instabilities in a clone of Nicotiana. — A Nicotiana clone, S5230-5, selected from cultures on interspecific hybrid origin is employed. Flowers of this clone show a high, spontaneous, somatic "mutation" rate (10^{-3}) for one heterozygous, genetic locus, and a much lower rate (10^{-5}) for another heterozygous locus. The phenotypic consequences of these instabilities are sectors of speckled and purple tissue respectively on the normally red petals. These sectors are counted per cm^2 petal area of estimated epidermal cell-number. — Clonal replicates were grown at Brookhaven throughout the summer (1953) at ten levels of chronic gamma irradiation (Co^{60}) varying from control to 300 r per day. Analysis of variance and graphical presentation are applied to data from samples of flowers scored during July and again during August. — The purple-sector frequency shows linear increase with dosage. However, the

regression of speckled-sector frequency is non-linear, and gives radiation-induced increments ten times those of the purple-sector frequency in the range below 12 r per day. Significant differences exist in speckled but not in purple sector frequency between clonal plants subjected to the same irradiation level. Mean frequencies obtained in August are about two-thirds those for July from the same plants. — In somatic tissue the speckled locus or a related physiological system is (1) hypersensitive to differences in gamma irradiation level at low dosage, and (2) also responsive to differences in other, potentially separable, external, environmental factors and/or to internal environment tentatively assumed to be correlated with metabolism and development.

SCHALET, A. and I. H. HERSKOWITZ, Indiana University, Bloomington, Ind. Chemical Mutagenesis of mature Drosophila sperm treated in sperm baths and postcopulatory vaginal douches.* — The present experiments were performed (1) as an independent test of the reproducability of the results of mutation studies in which mature sperm were treated by a nitrogen mustard administered in sperm baths and postcopulatory vaginal douches, (2) to test whether a change in solvent for the mustard would influence its mutability, and (3) to test other chemical substances for mutagenic action when applied by these methods. — The standard Basc technique with the Oregon-R stock was employed throughout for the detection of sex-linked recessive lethal mutations. Two series of experiments with 0.1% methyl bis (beta-chloroethyl) amine hydrochloride given by both methods were carried out by the junior author (Schalet), the earlier one giving 1.3% mutations (13 mutations in 973 tested X chromosomes) and the later one 2.5% (14 mutations in 555 tests). The latter value value approached the 3.6% rate obtained earlier by the senior author. No clear difference in mutation rate was noted in these experiments whether the solvent for the mustard was propylene glycol or a 0.62% saline solution. — Tests for mutations following treatments with sublethal concentrations of 20-methylcholanthrene (in sesame oil) or allyl isothiocyanate in propylene glycol were negative (1 lethal in a total of 542 tests). Results of tests of ethyl sulfate and other substances for mutagenic action will also be presented. (*This work has been supported by a grant received for work of H. J. Muller and and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

SHAPARD, PAULINE B. (introduced by M. M. Green), University of California, Davis, California. A physiological comparison of vermilion eye color mutants of Drosophila melanogaster. — The suppressed (v^s) and unsuppressed (v^u) vermilion mutants of D. melanogaster have been further studied and found to differ in several physiological characteristics. Both v^s and v^u fail to produce brown eye pigment under normal genetic and environmental conditions and both accumulate non-protein tryptophane at a level much higher than do v^+ individuals. Exogenously supplying either kynurenine or formylkynurenine to either mutant allows the production of brown eye pigment. The semi-dominant non-allelic suppressor gene sup-

presses both the eye color phenotype and the accumulation of non-protein tryptophane in v^s mutants, but has no apparent effect on either trait in v^u mutants. The eye color effect of the v^s mutants may also be overcome by partial starvation of larvae; it is not clear whether non-protein tryptophane accumulation is affected by "starvation." "Starvation" has no effect on the v^u mutants. That is, v^s mutants block tryptophane metabolism and, therefore, the production of brown eye pigment in a manner that can be overcome by specific genetic and environmental conditions, while no conditions have so far been found that will overcome the effects of v^u mutants, except supplying exogenously the normal products of tryptophane metabolism. It may be that v^s has an inhibiting action which is relatively easily destroyed, while v^u may more directly affect the production of the tryptophane oxidizing enzyme. This can only be determined through studies of the tryptophane peroxidase-oxidase enzyme system.

SINGLETON, W. R. and A. L. CASPAR, Brookhaven National Laboratory, Upton, N. Y. Effect of time of gamma radiation on microspore mutation rate in maize. — Corn plants were irradiated at different stages in the microspore development with 1300 r from a Co^{60} gamma source. The plants were grown in pails and moved into the gamma field where they were irradiated for 1 day only. The first plants were exposed just before meiosis, and the radiation program continued until pollen was shed, with a different group being exposed each day. — Some rather striking results were obtained. Meiosis was the most sensitive period for pollen injury, with at least 95% of the grains being injured. The same amount of radiation delivered 3 days later, and at all later dates, produced only about 10% damaged grains. — However, the time of greatest sensitivity for mutation production was fully 10 days later, just a few days before pollen shedding, when the rate for endosperm characters exceeded 3% per gene. More than 4,000 mutations were observed. — The average seed set was extremely low for meiotic irradiations, less than 20 seeds per ear. This rose quickly to a maximum of 200 seeds per ear, dropped to about 100 just before the period of maximum mutation production, then rose to 250 per ear at the period of maximum mutation production. The mutations dropped sharply for irradiations during the last 2 days before pollen shedding. — These data indicate that most of the mutations are produced at an entirely different time from maximum chromosomal damage as measured by injury to pollen. — Importance of these findings to plant breeding will be discussed.

SLATIS, HERMAN M., McGill University, Montreal, Quebec. Brown locus position effects in Drosophila melanogaster. — Position effects have been produced by irradiating both wild type and brown. A brown deficiency has a slight dominant effect on eye color. The 22 other brown rearrangements involve heterochromatin and are variegated. Homozygotes for R(+) position effects vary in pigmentation from phenotypes close to wild type to less than the heterozygote R(+)/bw. The heterozygote between two position effects is always moderately light in color. It appears that each position

effect has a characteristic intrachromosomal diminution of pigment and an interchromosomal effect and flies with two position alleles combine these effects. — Since the amount of pigment produced by interacting position effects appears to be predictable on a chemical basis, the structural theory of position effect, which explains gene action through the synaptic relationship of the chromosomes involved, is unsatisfactory. A simple chemical theory is proposed which assumes that brown is strongly affected by heterochromatin, and that the effect of heterochromatin is stronger on the chromosome which contains it than on the homologue. — Reinvestigation of the brown-dominant mutant brings it in line with other observations at this locus since it is found to be due to an insertion of heterochromatin very close to the brown locus. The heterozygote $\underline{bw}^{D}/+$ (and $\underline{st}/\underline{st}$) is always heavily variegated. Although the brown-dominant chromosome contains an excess of chromatin over the normal arrangement, in the homozygous condition it greatly reduces local crossing over. This has implications concerning various theories of crossing over.

SMITH, HAROLD H. and THORAYA A. LOTFY, Cornell University, Ithaca, New York. Comparative effects of beta-propiolactone and ceepryn (cetyl pyridinium chloride) on chromosomes of Vicia and Allium. — Root meristems of Vicia faba were treated for 1/2 hour with .02% (.0032 M) beta-propiolactone (BP1), .04% BP1, .001% (.000029 M) ceepryn (Cp), .002% Cp, .02% BP1 + .02% BP1, .02% BP1 + .001% Cp, .001% Cp + .02% BP1, and .001% Cp + .001% Cp. Combination applications were made 24 hours apart and fixations were made daily for one week after the first treatment. — Of the single-dose BP1 treatments .04% was the more effective and the average effect of the two concentrations was to give 13.5% cells with aberrant divisions. Of these 67% had fragments only, 24.5% bridges only, and the remainder various other aberrations. This is compared with the single-dose Cp treatments that produced 6.3% cells with aberrations of which 27.1% had fragments only and 71.2% bridges only. — In addition, a striking contrast in size of fragments was observed. BP1 treatments gave 81% large fragments, 16.2% medium and 2.8% small. Cp treatments gave no large fragments, 22.7% medium, and 77.3% small. Double dosages with either compound increased the frequency of smaller fragments, indicating additional sites of breakage. Combined treatments with both compounds showed different effects depending upon the order of application. — Single dosages with .02% and .04% BP1 were also used on root meristems of Allium cepa. Differences observed compared with Vicia were that .02% was more effective and the frequency of small fragments was much greater (52%). — The results are interpreted in terms of the difference in action of a cationic surface active denaturant (Cp) and a highly reactive alkylating agent (BP1); and the difference in distribution of heterochromatin within the chromosomes of Vicia and Allium.

SOKAL, ROBERT R., University of Kansas, Lawrence, Kansas. Selection for the separation of genetic correlates. — A genetic correlation has been established by Sokal and Hunter (1954) between resistance of larvae to

DDT in the medium and site of pupation in D. melanogaster. Selection for DDT—resistance brought about peripheral pupation of larvae, while selection for peripherality in pupation site resulted in resistance to DDT. Similarly, selection for susceptibility to DDT resulted in a central pupation pattern, while selection for centrality brought on susceptibility. — These earlier findings have been confirmed and extended. — Two new strains, derived from related stock, were started to test the possibility of breaking the above genetic correlation. The CR strain was selected simultaneously for centrality in pupation site and resistance to DDT, while the PS strain was selected for peripheral pupation site and susceptibility to DDT. Selection was necessarily from sibs on a family merit basis. Some 200 flies, representing offspring from 10 to 25 selected parent pairs were used as progenitors of each subsequent generation. — After 11 generations of selection some resistance had developed in the CR strain as compared with the PS strain. On the other hand no consistent trend in pupation site was apparent. The two strains alternated irregularly in peripherality. Thus there appears to be a conflict between the force of the genetic correlation tending to make the CR strain peripheral and the PS strain central, and the force of selection in the opposite direction. — This experiment lessens the likelihood of linkage between the two characters and makes a pleiotropic or some other physiological relation more plausible. A gene model based on this and other evidence is discussed.

SOOST, ROBERT K., University of California, Citrus Experiment Station, Riverside, California. Gene dosage effect of the Wo gene in tomato. — Tetraploid tomatoes were developed carrying various doses of the dominant lethal gene Wo. Plants with one, two, or three Wo genes were normal in growth and fertility. Plants with four Wo genes were not recovered. The phenotypic woolliness of the plants increased with gene dosage. The increase in woolliness is caused by an increase in the number of branched trichomes and an increase in the number of branches per trichome. The ave. number of trichomes per unit area was not increased. — Trisomics with one Wo gene had almost the same level of woolliness possessed by the Wo diploid. Trisomics with two and three Wo genes were not obtained. — The introduction of the Wo gene into four vigorous varieties of tomato did not increase the viability of the diploid Wo homozygotes.

SPARROW, A. H., V. POND and SELMA KOJAN, Brookhaven National Laboratory, Upton, New York. Microsporogenesis in excised anthers of Trillium erectum grown on sterile culture media*. — In order to study microsporogenesis under controlled conditions, a number of different culture media have been tested for their ability to support normal cytological development in anthers of Trillium erectum. Under sterile conditions, sets of anthers were removed from the flower bud at various stages of meiosis beginning as early as pachytene, placed on sterile culture media and examined periodically. Most of the anthers were allowed to go through to microspore division, then smeared and examined to determine the relative number of divisions and the degree of cytological abnormality (if any).

Eight different media were tested using a minimum of ninety-four anthers per medium. Disappointing results were obtained with all but two of the media. These were media consisting of basic nutrients used by Taylor (Amer. Jour. Bot. 37: 137-143) modified by the addition of two different concentrations of coconut milk. On these media more than seventy per cent of the anthers cultured at meiotic prophase reached the binucleate stage of microspore divisions in apparently normal condition. The results obtained so far definitely indicate that meiosis will proceed fairly normally in excised Trillium anthers grown on sterile culture media and that the method should prove very useful in experimental cytology. (*Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

SPOFFORD, JANICE B., University of Chicago, Chicago, Ill. The effect of expressivity on selection against eyelessness in Drosophila melanogaster. — The accumulation of modifiers that reduce the phenotypic distinctness from wild-type is frequent for certain mutant stocks, including eyeless (ey). That eye-size in itself is of selective importance in laboratory cultures is indicated by comparing offspring of crosses involving different degrees of expression of the same allele, ey^4. A nearly isogenic wild-type stock was derived from an outcross to a marker stock. Fourth chromosomes carrying ey^4 were introduced by use of the same marker stock, followed by inbreeding to insure that only one wild-type and one ey^4 fourth chromosome were present. The stocks were then perpetuated by mass transfers. Four grades of eyelessness were distinguished. When in competition with $+/ey^4$ at 25°C., larger-eyed ey^4 flies left more progeny than smaller-eyed ey^4 flies. In spite of the attempt to eliminate genetic heterogeneity in all save the fourth chromosomes, parent-offspring correlations indicate the segregation of modifiers. To avoid fixation of modifiers in the large and small grades of eyes, parents were selected from as many cultures with as diverse parental grades as possible. An increasing parent-offspring correlation in the course of the experiment suggested some accumulation of modifiers, whose numbers are estimated by a comparison of F_2 with F_1 variances for crosses between large- and small-eyed parents.

STEFFENSEN, DALE, Brookhaven National Laboratory, Upton, New York. Increased frequency of X-ray-induced chromosomal aberrations in Tradescantia produced by a mineral-nutrient treatment.* — Plants of Tradescantia paludosa (clone 5 of Sax) were grown in Hoagland's solution (full and 1/10 concentration) and in soil. The solutions were changed monthly for the first 6 months in culture and the pH became slightly basic just prior to each change. After this period the 1/10 concentrated solution was not changed for 3 months, during which time the solution became first basic and then strongly acidic (final pH of 4.3). Under these conditions a mineral imbalance was likely. At the close of this 3 month period inflorescences from all of these conditions were irradiated with soft X-rays (100 KVP) and were examined for chromosome aberrations

one and four days later. Chromatid exchanges were significantly higher in the buds from the depleted 1/10 concentrated Hoagland's solution than in those grown in either the regularly changed full concentration solution or in soil. The spontaneous aberration frequency of unirradiated plants grown in the depleted 1/10 concentration solution was no greater than that of the plants grown under either of the two other conditions. These results were verified in a second experiment. The depleted 1/10 concentration solution produced plants with more chromatid exchanges for each isochromatid and chromatid break observed than did the plants grown on the solution maintained at full concentration. These data can be interpreted to bear on the problem of chromosome restitution. (*Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

STRAUSS, BERNARD S., Department of Zoology, Syracuse University, Syracuse 10, New York. Studies on the Metabolism of Acetate by Acetate-Requiring Mutants of Neurospora crassa. — The ac mutants of Neurospora crassa require acetate for growth and accumulate pyruvic acid. Non-growing cultures shaken in buffer with glucose accumulate large quantities of pyruvate, cultures shaken in buffer with acetate accumulate smaller quantities of pyruvate. Studies using C-14 methyl or carboxyl labeled acetate indicate that pyruvate is formed from acetate by "leakage" from a tricarboxylic acid cycle. Sodium fluoracetate inhibits acetate and glucose oxidation in Neurospora and fluoracetate inhibited cultures accumulate citrate. ac mutants require up to 50mg of acetate for maximal growth under standard conditions. One strain (ac-4) will substitute about 40mg of succinate for acetate under special conditions. "Succinate" mutants (described by Lewis and by Dubes) give maximal growth on 2.5mg of succinate or on 2.5mg of acetate after a lag period. It is suggested that Neurospora crassa ordinarily uses the tricarboxylic acid cycle for acetate oxidation but that a dicarboxylic acid cycle becomes operative, adaptively, in the "succinate" mutants. — The amount of pyruvate accumulated by non-growing ac sp cultures shaken with glucose and buffer is reduced by the addition of acetate. This is a true inhibition of pyruvate formation by acetate. The significance of this inhibition of precursor formation by a product of the reaction chain will be discussed. (Work supported by contract AT(30-1) 1138 between Syracuse University and the Atomic Energy Commission.)

TASCHDJIAN, EDGAR, St. Francis College, Brooklyn. Genetics, Information and Communication. — This paper discusses the applicability of the concepts underlying Information Theory and Communication Theory to genetical problems, such as Mendelian heredity, linkage phenomena, mutations, degenerative inbreeding, excluding ontogenetic gene action. — Quantitative measures for information and entropies are calculated for several typical cases and it is shown that by considering the genotype as a set of symbols and heredity as a communication between parents and offspring, genetics can be profitably and more intimately connected with other disciplines.

TAYLOR, J. HERBERT, Columbia University, N. Y. Nucleic acid metabolism at the intracellular level. – Autoradiographs were prepared from larval salivary glands of Drosophila during mid-third instar after they had eaten food containing phosphorus-32. Since most of the P^{32}, remaining after extraction of acid soluble phosphates, was removed by ribonuclease, it will be referred to as RNA-phosphorus. No synthesis of DNA was detected at this stage. Resolution allowed detection of differential labeling in cytoplasm, chromatin and nucleolus. Quantitative comparisons were made by counting silver grains. – During the first hour labeled RNA appears in the chromatin and nucleolus, but cytoplasmic RNA is not detectably labeled. In two hours the RNA P^{32} per unit volume of cytoplasm is about one-fifth that in the nucleus. Larvae were removed to non-labeled food after two hours. During the next three hours the P^{32} in both nuclear and cytoplasmic RNA increased rapidly, but the rise in activity of cytoplasmic RNA is faster. Within five hours from the beginning of the experiment nearly uniform labeling of all parts of the cell is obtained. To the extent that incorporation of P^{32} represents synthesis of RNA, these experiments indicate a rapid synthesis in chromatin. Since the nucleolar activity also rises rapidly it must be a site of synthesis or rapid accumulation of material synthesized in the chromatin. The relation between rates of labeling of nuclear and cytoplasmic RNA is consistent with the view that most if not all of the cytoplasmic RNA is contributed by the nucleus and hence may represent the mechanism of gene interaction with the cytoplasm.

TELFER, J. D. and S. ABRAHAMSON, Indiana University, Bloomington, Ind. The higher egg mortality associated with insemination on the first than the second day after irradiation of Drosophila males. – Egg mortality was compared following ejaculation of Drosophila melanogaster sperm 0-24 and 24-48 hrs. after X-irradiation. Virgin adult males ($\underline{y}$ $\underline{In49}$ $\underline{f}$ $\underline{B}/\underline{sc^8}$.Y; bw^D/bw^D) 48-72 hrs. old were exposed to 3600 r and placed with equal number of virgin females (Y^s.X $\underline{InEN}$ $\underline{y}$.$\underline{Y^1}$;$\underline{st}$) for 24 hrs. The males were then given new virgin females for a second 24 hrs. Eggs were collected from the females of both groups every 12 hrs. for 4 days. In a total of 25,433 eggs scored, the survival rate (corrected for control mortality) for eggs of first day's insemination period was only $47.6 \pm 0.14\%$ but $71.4 \pm 15\%$ for those of second day. These results agree with those of Baker and Von Halle (1953). Further studies were undertaken to determine whether the lower survival rate for the first day's material was due to males' virginity (and possible sperm overripeness). Survival rate on using first post-treatment day's sperm of virgin males was $45.5 \pm 0.23\%$, while that of non-virgins was $49.7 \pm 0.24\%$. In both cases, second days' inseminations gave significantly higher survival. Additional identical control studies indicated that the observed differences are in zygotic mortality rather than in copulation frequency or effectiveness at different lengths of time after the disturbances associated with irradiation. It would follow that sperm ejaculated immediately following radiation are more susceptible to genetic radiation damage than those, apparently less mature at irradiation, which are

ejaculated a day later. This higher "dominant lethal" rate during the first 24 hrs. would be further augmented by "overripeness" if the males were not allowed to mate before irradiation. (Work has been supported by a grant to Dr. H. J. Muller and associates from the United States Atomic Energy Commission (Contract AT(11-1) -195).)

VERDEROSA, F. and H. J MULLER, Indiana University, Bloomington, Ind. Another case of dissimilar characters in Drosophila apparently representing changes of the same locus.* — Like vortex, oblique, and their alleles, which years after their discovery were unexpectedly found to be heteromorphic derivatives of what was shown to be probably one complex locus (Muller, 1921, Science 53: 97 et seq.), so too the sex-linked recessive small eye, sy, found by Bridges as a spontaneous mutant (Genetics of Drosophila, 1925) and by Muller (1928) as an X-ray-induced one, and the X-ray-induced recessive outstretched wings, od (Muller, 1930), arose separately and, having seemingly unrelated phenotypes, were not suspected of allelism, although Bridges (see Bridges and Brehme, 1944) had placed both at 59.2 on the linkage map. Abrahamson in 1953 discovered an X-ray-induced mutant, termed "odsy" by us, combining both these characteristics. Our crosses between these types showed each "compound" to exhibit that mutant characteristic, and only that, common to both parents. Thus, od/sy appears normal. Crossing over was studied in the presence of heterozygous inversions ("Curly" and "Payne") involving all major autosome arms, to promote crossing over. Females with "cis" ("coupling") arrangement, f car/odsy, gave no genuine crossovers among 17,498 offspring (apparent crossovers were tested), while those with "trans" ("repulsion") arrangement, f od car/sy, gave none in 9,714. Nevertheless the region f-odsy, ordinarily giving 2.5%, showed 4 and 5%, and odsy-car, ordinarily 3.3%, showed 10 and 13% crossing over in cis and trans crosses, respectively. Since it is very unlikely that both odsy and od or sy involve minute inversions, we conclude that these genes are alleles. We propose as their revised symbols os, os^{O}, and os^{S}. (*Work supported by U. S. Atomic Energy Commission grant (Contract AT(11-1)-195).)

VOLPE, E. Peter, Newcomb College of Tulane University, New Orleans, La. Mode of inheritance of the mottled pigment pattern in Rana kandiyohi. — Collections of meadow frogs from the southwest quarter of Minnesota and adjacent parts of South Dakota reveal occasional individuals which differ markedly in pigmentation from the common meadow frog, Rana pipiens. These chance individuals are characterized by black vermiculate mottling of the interspaces between the usual black spots of the common meadow frog. The correct taxonomic designation of this mottled meadow frog has been problematical. It has been variously listed as Rana kandiyohi, Rana pipiens kandiyohi, and Rana pipiens, phase kandiyohi. A series of breeding experiments have shed light on the problem. Six sets of cross-fertilization experiments involving the mottled meadow frog (provisionally regarded as Rana kandiyohi in the experiments) and the common meadow frog (Rana pipiens) were conducted. Each set consisted of the following

crosses: kandiyohi ♀ x kandiyohi ♂, kandiyohi ♀ x pipiens ♂, pipiens ♀ x kandiyohi ♂, and pipiens ♀ x pipiens ♂. Embryos derived from the crosses were reated beyond metamorphosis, and the results indicate that kandiyohi differs from pipiens by a single dominant gene. In the kandiyohi ♀ x kandiyohi ♂ crosses, the transformed tadpoles displayed a clear-cut phenotypic segregation into a statistically significant ratio of 3 kandiyohi to 1 pipiens. Hybrids between kandiyohi ♀ x pipiens ♂ (as well as the reciprocal cross) gave the expected 1 : 1 ratio. Only the typical pipiens pattern resulted from the pipiens ♀ x pipiens ♂ crosses. Rana kandiyohi should not have the taxonomic rank of a species or subspecies but should be reduced to synonomy with Rana pipiens and be referred to as the "kandiyohi dominant mutant".

WAGNER, R. P., Genetics Laboratory, Department of Zoology, University of Texas, Austin, Texas. An apparent metabolic block induced in a Neurospora mutant by threonine. — It has been previously reported that a Neurospora mutant, T77, grows well on minimal medium, but is inhibited by threonine at 35°C. It has now been found that in the presence of threonine, (but not in its absence), the slight amount of mycelium which does accumulate in the first 72 hours of growth produces a large amount of alpha-keto-β-ethylbutyric acid, a compound known to be a metabolic derivative of threonine and a precursor of isoleucine. Wild type does not accumulate this keto acid when growing in the presence of threonine, except briefly in the early phases of growth, after which time the compound rapidly disappears from the medium. Neither alpha-keto-β-ethylbutyric acid nor isoleucine are inhibitory to the growth of T77. — The significance of these findings in relation to the analysis of the metabolic disfunction which causes T77 to be inhibited by threonine will be discussed. — These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the University of Texas, Nonr 859(00).

WALKER, B. E. and F. Clarke Fraser, McGill University, Montreal, Quebec, Canada. The embryological basis for a strain difference in susceptibility to a teratogenic action of cortisone. — A particular schedule of cortisone injections into pregnant mice produces cleft palates in 100% of the offspring of A/Jax strain ♀♀ and in 18% of the offspring of C57BL strain ♀♀ (Kalter, Genetics 39: 185). The present study has shown that the secondary palate normally closes at a later stage of embryonic development in strain A/Jax than in strain C57BL. Cortisone treatment causes some delay in C57BL embryos and great delay in A/Jax embryos in the sliding of the palatine shelves from a vertical to a horizontal position. The palatine shelf movement was shown, by observations on fixed material and in vivo, to depend on a force that is built up within the shelves. The time at which the palate closes appears to depend on the rate of increase in this force. A strain difference in the ability of the maternal-embryonic system to build up the force necessary to bring about palatine shelf movement is considered to be the primary basis for the strain difference in reaction to the teratogenic effect of cortisone. (Work supported by the Banting Research Foundation.)

WHITING, P. W., University of Pennsylvania, Philadelphia, Pa. An impaternate gynander of the wasp Neralsia. — From parasitized blow-fly maggots collected at Woods Hole, Mass., August 1949, there were bred 48 females, 10 males of N. armata (Say) (Cynipoidea, Figitinae) (Identified by Lewis H. Weld). A second generation maturing in October and a third in December totalled 356 females and 183 males from females exposed to males; 1131 males (arrhenotoky) and one gynander (impaternate) from unmated females. The gynander (about 5 mm. in body length) had female abdomen and right antenna (11 short flagellar segments), male left antenna (12 long flagellar segments). — Males were more variable (4 to 6 mm.) in body length than females (4.5 to 5.0 mm.). Selection of large and small specimens did not affect number of flagellar segments. In female antennae these were always 11 in number and short, the terminal enlarged; in male antennae these were 12 and elongate. — The parasitized maggots, collected as they left the meat, formed pupae with legs and wing-pads well-developed and pigmented, but eyes colorless and general body surface smooth and white without bristles. Pupae were not formed, however, by infected maggots of the larger species (Calliphora, Sarcophaga). These died when half-grown and the parasite larva pupated within the partially consumed host. In no case were more than one wasp found within a single puparium of the many that were opened.

WOLFF, S. and R. C. VON BORSTEL, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. The effect of pre- and post-irradiation centrifugation on the chromosomes of Tradescantia and Vicia. — Inflorescences of Tradescantia were irradiated with 200-r X rays. If the inflorescences were centrifuged within 30 seconds after the cessation of irradiation, the number of two-hit chromosome aberrations was increased. This is explained as being due to the physical separation of broken chromosome ends which consequently reduced the amount of rejoining. Similar results could be attained with as much as a 10-minute interval between irradiation and centrifugation if the plants were kept at 7 °C. In order to prevent rejoining in this period of time. — If on the other hand, the plants were centrifuged previous to irradiation, the numbers of two-hit aberrations were reduced by a factor of two. At present we attribute this to a centrifugal "packing down" of the chromosomes which is not cytologically detectable. This "packing" would restrict chromosome movement and hence decrease the probability of exchange formation. — Similar experiments have been performed on the seed of Vicia faba.

WOODWARD, Val W., Brookhaven National Laboratory, Upton, L. I., N. Y. Mutation Rates at One Glutamic Acid Locus in Neurospora crassa. — It is possible, by means of the previously reported filtration and selective plating technique, to recover biochemical mutants of Neurospora in quantities sufficiently large to facilitate determinations of spontaneous and induced mutation rates. Previous studies of gene mutability and mutation rates in Neurospora have utilized the back-mutation technique. Since the mechanism and rates of back-mutations may differ greatly from those of

"forward" mutations, it is important to be able to study the characteristics of both types. — The macroconidial strain, 74A-3b, was used as a stock culture. Most conidia of this strain contain 1-3 nuclei (as many as 8 have been counted in a few conidia); therefore, the mutation rates are observed for numbers of mutant conidia rather than mutant nuclei. However, knowing the nuclear distribution, mutation rates per nucleus can be calculated. — The number of mutants per 10^4 conidia recovered from untreated and irradiated populations were as follows: untreated, 0.8; 4,560r x-ray, 5; 12,540r x-ray, 11; 21,600r x-ray, 18. A total of 66 mutants were recovered. These data indicate a linear dose-mutation relationship. (The author is a National Institutes of Health Post-doctoral Fellow. Research carried out at Brookhaven National Laboratory under the auspices of the United States Atomic Energy Commission.)

YANDERS, A. F., Northwestern University, Evanston, Illinois. An influence of age at time of treatment on the induction of Minute effects in the sperm of Drosophila melanogaster. — Adult males of D. melanogaster (Oregon-R) were exposed to 4500 r of X rays at one, seven, or fourteen days after eclosion. Each male was immediately pair-mated, then transferred to a new female at twenty-four-hour intervals for one week. The occurrence of dominant Minute characteristics, believed to be due to small deletions, was observed in the progeny. The frequency was highest in the oldest group, intermediate in the middle group, and lowest in the youngest group. The mean percentage of Minutes on successive days after irradiation showed a characteristic pattern in each age group. Curves of these values exhibit a striking similarity in form to those plotted for induced dominant lethals (from the data of Lüning, Hereditas 38: 91-107, 1952), suggesting that similar mechanisms are responsible for both dominant lethals and Minutes. This lends further support to the hypothesis that sperm increase in susceptibility to irradiation with increase in the age of the irradiated male, probably because of increased chromosome breakability. (Supported by a research grant to Dr. G. H. Mickey from the United States Atomic Energy Commission, Contract No. AT(11-1)-89, Project No. 7.)

ZAMENHOF, S., G. LEIDY and E. HAHN, Columbia University, New York, N. Y. Unstability of transforming principle induced by mutagenic agents. — The transforming principle (DNA) of Hemophilus influenzae is stable to heating (76°, 1 hr), incubation (37°, 22 hrs) or storage (6°, $\frac{1}{2}$ year), as evidenced by unchanged transforming activity and viscosity. When the decrease of activity and viscosity on prolonged heating was studied in function of time, the curves obtained suggested that more than one reaction is involved (such as unstabilization followed by inactivation). When the transforming principle was heated at 76° for more than 1 hr, the surviving active molecules became increasingly unstable to heat, incubation or even storage. Similar unstabilization of active molecules was obtained by sublethal action of U.V., nitrogen mustard (HN2), desoxyribonuclease and pH change. — The unstabilization of heredity determinants (transforming

principle, DNA) by mutagenic agents in vitro may have a bearing on the frequently inferred unstabilization of genes prior to actual mutation and on the delayed effect of mutagenic agents. — When the transforming principle was heated for 1 hr at 92° and the surviving 1 per cent, now rendered very unstable, was used for transformation experiments, the transformed cells yielded transforming principle which was completely stable: thus, the change (unstabilization) in vitro was not retained on reproduction and therefore was not a "mutation in vitro." As this change probably involves random breaking of hydrogen bonds in DNA molecule, it appears that such random breaking per se is not a basis of mutations. (This work has been supported by the Public Health Service grants, H. E. Alexander and S. Zamenhof, principal investigators.)

SAEZ, F. A. and M. E. DRETS, Departamento de Citogenética, Instituto de Investigación de Ciencias Biológicas, Montevideo, Uruguay. Cytological Alterations Induced by "Gonyleptidin" during Cell Division. — The action of Gonyleptidine (1), a new volatile substance of animal origin with antibiotic properties, was studied. Concentrations of 1-1,000 gammas per c.c., during varying times of action and recuperation, were used. — Meristem cells of Allium cepa roots show alterations in the helicoidal cycle (different degrees of linear contraction), diplochromosomes, delayed division of the centromere, C-metaphases, polyploidy, blocking of mitosis and interchromosomal coalescence of varying degrees. — Conspicuous findings are: Chromosome fragmentation and pulverization, with diffusion of DNA to the cytoplasm and concomitant loss of nuclear stain intensity. — In Germ cells of Laplatacris dispar (Orthoptera) different degrees and forms of coalescence even mass agglutination, were found. An important fact is the behaviour of the sex chromosome, which never agglutinates with the autosomes. Occasionally, chromatin rarefaction, appearing as holes distributed along the chromosome length, was observed. — Metaphase I is quickly affected by Gonyleptidin, and the chromosomes are deformed showing as Feulgen positive spherical bodies lying on a cytoplasmic background slightly stained by this reagent. In extreme cases, chromosomes agglutinate in one single mass. — Polyploid giants cells, reaching sizes up to 10 times the normal are often found. Gonial metaphases, as well as meiotic prophase, metaphase and anaphase I, presented a high degree of polyploidy. — In the diplotene stage., multivalent associations, and also configurations denoting the existence of different kinds of structural alterations, (high number of chiasmata, bridges, fragmentations, translocations, etc.) were observed. — Apparently normal zones were found beside affected ones in both plant and animal material. — These findings demonstrate the Gonyleptidin has a general physiological action, interferring with cell metabolism, disturbing and inhibiting growth and cell division. — On account to the Dariophysiological effects with indubitable genetic meaning, it is concluded that Gonyleptidin is a substance endowed with mutagenic properties. — (1—This substance was discovered by Prof. C. Estable at the Instituto de Investigación de Ciencias Biológicas de Montevideo.)

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A PERIODICAL RECORD OF INVESTIGATIONS BEARING ON HEREDITY AND VARIATION

Founded in 1916

by

GEORGE H. SHULL

with the cooperation of

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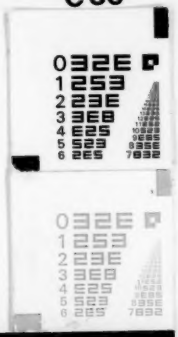
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